NASA Technical Memorandum 88379

1984-85 NASA Space/Gravitational Biology Accomplishments

DECEMBER 1985

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1984-85 NASA Space/Gravitational Biology Accomplishments

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**Scientific and Technical
Information Branch**

1985

Preface

The Space/Gravitational Biology Program currently includes forty-one research tasks. Individual technical summaries of each of these projects are presented in this publication; the summaries consist of a description of the research, a listing of the project's accomplishments, and an explanation of the significance of the accomplishments. The summaries cover the period from March 1984 through March 1985.

The intent in compiling this publication is twofold. First, we wish to provide the scientific community with an annual summary of the accomplishments resulting from research pursued under the auspices of NASA's Space/Gravitational Biology Program. Secondly, we hope to stimulate an exchange of information and ideas among scientists working in the Program. To facilitate this exchange process, a list of publications has been included with each task summary. Accomplishments of particular significance in each task have been underlined.

We would like to thank all of the participants in the Space/Gravitational Biology Program for their cooperative response to our requests for information. We would also like to thank April Commodore Roy and Janice S. Wallace for their technical assistance in the preparation of this report.

Thora W. Halstead
October 1985

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INTRODUCTION

THE NASA SPACE/GRAVITATIONAL BIOLOGY PROGRAM

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Introduction

One of the major features of the physical environment on the surface of Earth is the constant presence of the force of gravity. Terrestrial gravity has important biological consequences for organisms living on Earth. The phenomenon of weightlessness which is encountered on spacecraft provides an excellent biological research opportunity, both because of its uniqueness to space and because of the importance of gravity to life on Earth. Access to space provides an opportunity to manipulate gravity from its norm of one down to almost zero, effectively providing the full spectrum of gravitational research capability for the first time. This capability, combined with the stability and pervasiveness of gravity on Earth, its obvious impact on biological evolution, and its continuing effect on the morphology, physiology, and behavior of living organisms, has led the Space/Gravitational Biology Program to concentrate its efforts and resources on investigating the biological significance of gravity.

Program Goals

The goals of the Space/Gravitational Biology Program are to: use the unique characteristics of the space environment, particularly microgravity, as a tool to advance knowledge in the biological sciences; understand how gravity has shaped and affected life on Earth; and understand how the space environment affects both plant and animal species, thereby enhancing our capability to use and explore space.

Program Scope

Research in the Space/Gravitational Biology Program is divided into three broad areas:

1. Gravity perception. The objectives are to identify gravity receptors in organisms sensitive to gravity and determine their structure and function, and to elucidate the mechanisms by which gravitational stimuli are perceived and transmitted to a responsive site.

2. Developmental biology. The objectives are to determine the effects of gravity, and especially weightlessness, as provided by spaceflight, on the genetic integrity, cellular differentiation, reproduction, development,

growth, maturation, and senescence of living systems; and to examine the evolutionary importance of gravity as a determinant of the form and function of terrestrial life.

3. Biological adaptation. This area includes the use of gravity's physiological effects to explore biological problems; and achievement of an understanding of how gravity affects and controls the physiology, morphology, and behavior of organisms, of how gravity and other environmental stimuli and stresses interact in this control, and of the biological mechanisms by which living systems respond and adapt to altered gravity, particularly that of the space environment.

Research Opportunities

With the proven feasibility of the Space Shuttle, we now have a new capability of performing biological experiments in space. The opportunity has arrived to use the locker space within the Shuttle orbiter on a continuing space available basis. This will provide a valuable augmentation to the ongoing ground-based research program.

Spaceflight will provide the validation for many experimental hypotheses developed in ground-based research, while gravitational experiments on Earth will continue to hone the questions, provide the necessary baseline data, and develop spaceflight experimental protocol.

The experimental approach of the ground-based studies in the Space/Gravitational Biology Program is to manipulate gravity on Earth and develop weightless simulation models to: (1) develop and test gravitational hypotheses, (2) identify gravity-sensitive biological systems and interacting environmental response mechanisms, (3) analyze biological systems and mechanisms known to be gravity-sensitive, (4) analyze flight experiment data and iteratively expand ground research capability, and (5) plan and design future space experiments. In addition, research is conducted to understand how the uncontrollable biodynamic factors of the spacecraft will affect the results of the various flight experiments.

Focus of Program

The research focus of the Space/Gravitational Biology Program is dependent upon several dynamic factors: the requirements of NASA, the characteristics of flight experiment opportunities, the sensitivity of specific biological systems to gravity, the scientific value of the research, the state of knowledge and technology in the specific scientific areas, the interest of scientists in studying the biological questions, and the availability of funds to support the research.

Within the scope of the Space/Gravitational Biology Program, the current Program is focused on answering the following basic scientific questions:

1. What are the components of the gravity-sensing mechanisms of plants and animals? How do they perceive information? How is the information transmitted to evoke responses?

2. Does gravity influence fertilization and development of plants and animals, and can fertilization and development proceed normally in a near zero gravity environment? If gravity does affect fertilization and development, what are the sensitive physiological systems and how are they affected? If early development is affected by gravity, is it a result of an effect on the parent or a direct effect on the embryo itself?

3. What is the role of gravity in the formation of structural elements such as lignin, cellulose, silica, chitin, and bone calcium phosphates at the molecular level as well as at more complex organizational levels?

4. What role does gravity play in calcium-mediated physiological mechanisms and in calcium metabolism?

5. How does gravity as an environmental factor interact with other environmental factors to control the physiology, morphology, and behavior of organisms? Or, how do gravitational and other environmental stimuli interact in the control and direction of living forms? Can the action of gravity be replaced by different stimuli?

As the new opportunity to conduct biological research in space grows, an increasing amount of the research supported by this Program will contain at least some results obtained in space. The future of the Program is tied to space research.

ACCOMPLISHMENT HIGHLIGHTS

ACCOMPLISHMENT HIGHLIGHTS

Below is a list of selected accomplishments of particular significance identified during the past year. More detailed information about each can be found in the individual technical summary reports that follow.

PLANT

- Amyloplast sedimentation has been timed in corn roots and shoots and been found to occur well within the presentation time (4 min).
- Amyloplasts have both ATP-dependent and -independent pathways for calcium uptake, and can sequester calcium when supplied with phosphate.
- Calcium enhances gravity-induced transport of auxin and determines the effectiveness of auxin as a growth suppressor in roots.
- Evidence indicates that calmodulin is an important regulator of root gravitropism.
- The steps: lateral transport of auxin $\longrightarrow$ proton gradient across cell wall $\longrightarrow$ asymmetric cell elongation have been confirmed as the sequence occurring following g stimulation of plant shoots.
- The gibberellin hormones regulate gravitropic curvature in oat shoots.
- Plants grown for multiple generations on a clinostat exhibit a delayed appearance of reproduction and a lowered reproductive productivity. More vegetative plant parts (roots, leaves, etc.) are produced because of this delay.
- Gravitropic stimulation selectively affects the sensitivity of plant stem cells to auxin.
- Gravitropic stimulation of plant stems does not alter cellulose microfibril orientation or internal cell pressures.
- The gravitropic response occurs simultaneously on both the top and bottom of the stem. The upper surface undergoes a small contraction while the lower surface doubles its growth rate.
- Mechanical stress to plants, induced through seismic manipulation, reduces plant growth, enhances plant strengthening, alters auxin transport, and inhibits auxin and gibberellin-type activity.

- Circumnutation of a plant's stem was shown to continue in the absence of a significant g force in spaceflight.

ANIMAL

- Organic matrix of rat otoconia is similar to that of the neogastropod shell and fish graviceptor.
- Organic material is important in the seeding and growth of otoconial crystals.
- Organic matrix of rat otoconia does not diminish during maturation of the fetus to adult.
- Anatomical evidence suggests that information concerning linear acceleration is processed peripherally, beginning at the hair cell level before being sent to the central nervous system.
- Significantly fewer statoliths are synthesized in the gravity receptor of developing jellyfish during clinostat rotation, suggesting a role for gravity in their normal development.
- Clinostat-rotated mouse eggs mature normally and can be fertilized, suggesting that late stages of egg maturation and subsequent fertilization will not be directly affected by spaceflight microgravity.
- Musculoskeletal development during mouse embryo maturation is sensitive to hypergravity (1.8-3.5 g).
- The same stages in limb development are sensitive to both hypergravity and known teratogens.
- There was an apparent retardation of vestibular system development in rat embryos during prenatal development on Cosmos 1514.
- The reproductive capacity of rats is more sensitive to hypergravity (1.3-2.1 g) than that of mice, which are smaller animals, indicating a proportional responsiveness to gravity depending on body size.
- Muscle loss in rat hindlimbs during simulated weightlessness causes the remaining muscle mass to fatigue more readily and to develop an increased sensitivity to hormones that regulate muscle mass.
- Four steps were found to be essential in the activation of bone-forming cells. One step appears to be weight-bearing or gravity-dependent.

- Body temperature regulation is optimal at 1 g. It drops at increased or decreased g levels.
- Body temperature is reset whenever gravity load is changed.
- A gravity-induced reset temperature has increased sensitivity to other environmental stresses.
- Squirrel monkey studies suggest that circadian rhythms for sleep, temperature, feeding, and drinking are lengthened during hypergravity exposure.
- The vitamin D metabolite 1,25-dihydroxy vitamin D, which regulates bone mass, was found to decrease in rats within the first week of suspension as a result of (rather than the trigger for) the suppressed bone growth.

PLANT PROJECTS

LOCALIZE AND IDENTIFY THE GRAVITY-SENSING MECHANISM OF PLANTS

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Description of Research

This laboratory has developed a working theory for the mechanism of sensing and transduction of the gravitational stimulus. The basis postulates of the theory are: (a) Gravity causes the settling of dense cell organelles; (b) the organelles carry a fixed charge and, in settling, cut and deform the cell's bioelectric field; (c) this deformation results in transient potential changes across cell membranes such that; (d) voltage-gated channels in the plant's vascular tissue open and/or close, resulting in; (e) alterations in the amount of calcium and potassium in the cells; (f) this steepened ion gradient amplifies the initially weak field deformation; (g) resulting in an asymmetric distribution of the plant growth hormone, indole-3-acetic acid (IAA) and, ultimately, asymmetric growth. We refer to this theory as the potential-gating theory of geo-sensing.

Portions of this theory (postulates 1 and 2, for example) are old and well documented. The impetus for the newer portions of the theory lies (a) in our finding that the free IAA of the mesocotyl of a corn shoot is concentrated in the vascular stele, whereas ester IAA is concentrated in the cortex; and (b) that, following a gravitational stimulus, both free and ester IAA increase in the mesocotyl cortex. This finding means that enhanced hydrolysis of ester IAA in the cortex, such as had previously been postulated by us, could not account for the increase in both free and ester IAA, but could only account for changes in the ratios of free to ester IAA. Thus, we concluded that the gravitational stimulus most probably caused increased "leakage" of IAA from stele to cortex.

The postulates concerning bioelectric potentials are based upon earlier studies, primarily those of E.J. Lund in his classic work, "Bioelectric Fields and Growth." The concept of amplification of field deformation by ion movement results from a need for a mechanism for amplification of the extremely weak currents generated by movements of charged particles in the bioelectric field. Finally, the notion of voltage-gated channels for "leakage" of ions and hormones stems, in part, from analogy to the gated channels of the much better studied animal neurons, and, in part, from studies in this laboratory on transport of IAA from kernel to shoot.

Accomplishments

(1) The major accomplishment is the development of a coherent and testable theory for the sensing and transduction of the gravitational stimulus. This theory, for the first time, integrates known effects of gravity on calcium and potassium movements, as well as data on hormone asymmetries and the resultant growth asymmetries.

(2) The primary experimental result was the finding that the gravitational stimulus induces an asymmetric distribution of both free and ester IAA. This finding is based upon assay of the endogenous ester and free IAA, and also upon the distribution of radioactivity in the shoot following application of 5-[^{3}H]-IAA or 5-[^{3}H]-IAA-myo-inositol to the seed.

(3) An additional experimental finding was that the IAA transport inhibitor, N-1-naphthylphthalamic acid (NP), increased the loading of 5-[^{3}H]-IAA from the seed into the shoot. It was this finding which first suggested that the hormone was moving in a symplast in which the plasmodesmatal connections could be gated.

(4) It was further found that calcium decreased movement of IAA from seed to shoot, whereas it is known to increase movement of IAA from the tip of the plant to the base. Thus, NP and calcium exert effects on the upward transport of IAA which are opposite to those exerted on its downward movement.

(5) We have chemically characterized a major catabolite of IAA as the 7'-0-glucoside of 7-hydroxy-oxindole-3-acetic acid. This hitherto undescribed metabolite represents step 3 in a new pathway for the catabolism of IAA.

Significance of the Accomplishments

The development of the potential gating theory of geo-sensing, together with our expanded knowledge of the reactions which determine the steady-state concentration of growth hormone in the plant, will lead to new experiments and, ultimately, we hope, to improved plant productivity both on Earth and in space.

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RESEARCH IN GRAVITATIONAL PLANT PHYSIOLOGY

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Description of Research

Among the some 20 or 30 fundamentally different environmental factors that have the most important effects on organisms, the force of Earth's gravity must rank near the top. To study how gravity is important to plants, we need to manipulate experimentally both the direction and magnitude of the g vector. In our Earth-based laboratories, our means to make g an experimental variable are strictly limited.

Numerous scientific questions remain to be answered, since we do not understand very well how plants detect so precisely the vector direction of the g force, how they measure its intensity, how they sum their g information (environmental stimuli) and store (remember) it, how they forget it, and how they use it to initiate biologically significant processes of growth and physiological behavior.

To address such broad questions, we employ centrifuges to study specific plant responses to acceleratory forces greater than Earth's 1 g. With these devices, centripetal acceleration can be accurately controlled--in our studies, over a continuous range from 1 to 20 g. We also use various configurations of slowly rotating machines called clinostats to simulate g levels in the hypogravity range ($0 < g < 1$).

We have used the NASA Shuttle to test predictions of how specific plant processes will be influenced by protracted g forces below 1 g, including weightlessness or microgravity (μg). To produce a desired hypogravity environment above μg, an onboard centrifuge must be used.

These devices on Earth and in space enable us to explore biological effects over a full "spectrum" of g levels from zero to 20 g. Of special interest is the opportunity we have had to compare quantitatively the effect of some hypogravity level achieved by simulation in our Earthbound laboratory with the effect produced by true μg in Spacelab. In that way we can validate, or discredit, the clinostat simulations we and others have depended upon for the development and testing of theories that attempt to explain how gravity is important to plants.

Accomplishments

In the past year our ground-based laboratory research has become even more closely related to our flight experiments. Development of the HEFLEX Experiment, which was carried out on Spacelab (SL)-1, was heavily indebted to our supporting work with clinostats and the UCSC Plant Centrifuge. The HEFLEX Experiment had as its principal scientific objective the testing of whether circumnutation of the hypocotyls of 4-day-old sunflower seedlings would persist in the absence of a significant g force. HEFLEX data demonstrated convincingly that circumnutation proceeded in μg; that qualitative question was therefore answered unambiguously. However, laboratory tests prior to the SL-1 Mission had provided us with data on circumnutational parameters at 1 g and throughout the full range of simulated hypogravity, $0 < g < 1$. We could make quantitative comparisons of the effects of protracted μg, clinostat-simulated zero g and 1 g, and we discovered that growth oscillations were much more vigorous in space than on the horizontal clinostat. We would not have been surprised had we found the reverse--little or no circumnutation in space compared with reduced but still significant oscillations on the clinostat--because, like so many physiologists who study effects of simulated weightlessness, we know that simulations are less than perfect. The surprising result we did observe is clear evidence that, at least for hypocotyl circumnutation, the clinostat was not a valid simulator for the free fall condition. No doubt this serendipitous discovery will encourage investigators of other phenomena to distrust the implications of experimental results from clinostat experiments and should generate a renewed appreciation of the importance of verifying the applicability of clinostatting by direct comparison of results from clinostat experiments and those from spaceflight. We may note also that one can cite no better example than this of the synergistic relationship between studies conducted in space and those performed on the ground.

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THE ROLE OF GRAVITY IN APICAL DOMINANCE

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Description of Research

Inversion of the upper main shoot of the herbaceous plant, Pharbitis nil (Japanese Morning Glory), induces vigorous outgrowth of the otherwise inactive lateral bud adjacent to the bend in the stem within 24-36 hr. The long-term objectives of this research have been (a) to determine how shoot inversion causes the highest lateral bud to grow out, and (b) to elucidate the interaction between gravitational forces and the regulatory mechanisms of bud outgrowth on a precise chemical basis. The short-term objective during the 1984-85 year has been to determine whether or not the plant hormone ethylene plays a role in shoot inversion induction of lateral bud outgrowth, and, if so, to elucidate that role. Experiments were carried out as follows: In plant shoots which were positioned either upright, inverted, continually rotating, or were mechanically perturbated, analyses were done on (a) growth of the main shoot and lateral bud, (b) ACC synthase activity (ACC synthase is the key enzyme in ethylene biosynthesis) and ethylene production, and (c) effects of ethylene inhibitors.

Accomplishments

Results of experiments demonstrate that:

(1) Shoot inversion has no effect on endogenous ethylene evolution in the highest lateral buds (or their nodes) in the upright portion of the shoot.

(2) Direct treatment of active and inactive lateral buds with ethylene promoters or inhibitors has no effect on bud outgrowth.

(3) The presentation time for shoot-inversion induction of ethylene production is about 2 hr.

(4) The treatment of the inverted portion of the shoot with the ethylene action inhibitor, $AgNO_3$, dramatically eliminates both the restriction of shoot growth and the promotion of lateral bud growth which usually accompany shoot inversion.

(5) The treatment of the upper shoot of an upright plant with ethrel (an ethylene-releasing compound) mimics shoot inversion by retarding upper shoot growth and by inducing outgrowth of the lateral bud basipetal to the treated region.

(6) Shoot inversion-induced ethylene indirectly promotes outgrowth of the highest lateral bud by restricting shoot growth.

(7) In simulated gravity nullification, where plants are rotated on a clinostat about a horizontal axis, ACC synthase activity and ethylene production are decreased and the usual inhibition of main shoot growth is negated. Lateral bud growth

does not occur.

(8) Mechanical perturbation of stem indirectly promotes outgrowth of the lateral bud below the affected stem region by restriction of growth of the upper main shoot (of an upright plant) via increased ACC synthase activity and ethylene production.

Significance of the Accomplishments

Finding #1, that inversion of the upper shoot has no effect on ethylene production of buds (or adjacent nodes) that will subsequently elongate, suggests that ethylene has no direct promotive effect on bud growth. Likewise, Finding #2, that ethylene promoters or inhibitors have no effect when applied to inactive lateral buds or to induced (by inversion of the upper main shoot or by decapitation) lateral buds, confirms that ethylene does not control lateral bud outgrowth by direct action on the bud. Previously we had shown that the beginning of inhibition of main shoot growth (<24 hr) following upper shoot inversion precedes the beginning of lateral bud outgrowth (24-36 hr). The Finding #3, of a presentation time for shoot-inversion induction of ethylene production of 2 hr is consistent with this result. These findings, and the fact that the treatment of the inverted portion of the shoot with the ethylene inhibitor, $AgNO_3$, dramatically eliminates both the restriction of shoot growth and the promotion of lateral bud growth which normally accompany shoot inversion (Finding #4) strongly suggest a cause and effect relationship between the restriction of main shoot growth and outgrowth of the lateral bud. Likewise, the mimicking of this shoot inversion effect by the application of ethrel (which releases ethylene) to the upper shoot of an upright plant (Finding #5) further supports the hypothesis (Finding #6) that shoot inversion-induced ethylene indirectly promotes outgrowth of the highest lateral bud by restricting shoot growth.

The Finding #7 that the nullification of gravity effect of shoot inversion by continual rotation of the plant on a clinostat also nullifies ACC synthase activity, ethylene production, and restriction of shoot and promotion of lateral bud growth provides further evidence that it is the ethylene generated from shoot inversion that is responsible for main shoot growth restriction. Mechanical perturbation (rubbing of stem with thumb and forefinger 15 times twice daily) is another way (in addition to shoot inversion) to generate ethylene evolution. The fact that this also results in restriction of main shoot growth and the promotion of subsequent outgrowth of the lateral bud (Finding #8) provides further support for the hypothesis presented in #6.

In summary, the results indicate that ethylene plays an indirect role in shoot-inversion promotion of lateral bud growth. These data also provide a framework for asking more questions. What is the role of gravity in the enhancement of ethylene production? Is ethylene production stimulated by amyloplast pressure on the ceiling of the inverted cell or by the accumulation of auxin via

gravity inhibition of auxin transport? How does ethylene cause restriction of shoot growth and how does this result in lateral bud outgrowth? The answers to these questions will bring us closer to the accomplishment of our long-term objectives.

BIOPHYSICAL MECHANISM OF DIFFERENTIAL GROWTH DURING GRAVITROPISM OF STEMS

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Description of Research

When a young plant is placed in a horizontal position, within minutes the stem begins to grow upwards. This reorientation of the stem occurs by differential growth on the two sides of the stem. The lower side grows faster than the upper side, causing the stem to bend upwards. The aim of this research is to elucidate and quantitate the biophysical basis for these changes in growth rate.

Work in 1984 was directed at defining the growth response of a plant suitable for further studies with the pressure probe. Dark-grown cucumber seedlings (Cucumis sativus L.) are very sensitive gravitropically, curving 90° in 45-60 min. This is among the fastest reported rates of curvature. The growth response to gravitropic stimulation was studied with seedlings marked at 2-mm intervals and photographed at 15-min intervals.

Accomplishments

(1) Software for image-analysis of the marked plants was improved and extended. In particular, programs were constructed to sum the responses of a series of plants and to display the data in several different ways.

(2) From the averaged responses of 20 plants, it appears that:

- (a) curvature starts in less than 15 min after stimulation;
- (b) both sides of the stem react simultaneously: the upper surface ceases expansion entirely, while the lower surface more than doubles its growth rate;
- (c) at the peak rate of curvature, the upper surface undergoes a small but real contraction; at the same point in time, there is a 25% per hour differential in growth rate across the stem.

(3) From the following experiment, it appears that the contraction on the upper side of the stem is a passive process, caused by shear deformation of the bending stem. When the growth on one side of the cucumber stem is stimulated by local (unilateral) auxin application, the stem begins to bend. The opposite side is only slightly affected: the rate of expansion is reduced about 3% per hour. This result shows that shear forces generated during stem bending can cause small but real compressive strains on the inner concave surface.

(4) In preliminary experiments with angular displacement

transducers resting on the apex of the horizontal stem, the lag before the start of curvature was found to be about 12 min.

(5) A novel in vivo stress relaxation technique has been developed and tested using pea (Pisum sativum L.) seedlings. The methods permits the most direct measurement of the apparent cell wall extensibility and the yield threshold for growth. Auxin-stimulated growth was used to test and calibrate the method. The results show that the rapid stimulation of growth by auxin can be completely and quantitatively accounted for by increase in cell wall extensibility.

Significance of the Accomplishments

The quantitative description of the growth response to gravitropic stimulation (Findings #1, 2) provides the essential background for biophysical studies on the mechanism(s) of this response. Two points are important in this respect. First, the response begins simultaneously in all parts of the growing stem. The change in growth does not moves as a wave, starting at the apex and moving towards the base. This is quite different from the growth pattern of certain phototropisms, and suggests that different mechanisms may be involved in the two types of tropisms. Second, the asymmetry in growth rate at the point of maximum rate of curvature is about 25% per hour. This is a very large asymmetry, and accounts for the rapid curvature. No mechanism has yet been shown to account in a quantitative way for such a large growth asymmetry.

Interpreting the apparent growth rates on the two sides of a curving organ requires some caution because the two sides are mechanically linked to one another. Passive shear forces generated by bending can cause compression on the inner side of the curving organ. The auxin experiments (Finding #3) showed that shear forces can account for only a small part of the response of the upper surface of the gravitroping stem. The simplest interpretation is that gravitropic stimulation inhibits growth completely, but transiently, on the upper stem surface. The small contraction is due to shear-generated compression, which is superimposed on the cessation of growth.

The stress relaxation technique (Finding #5) provides a way to measure cell wall properties without complications due to water transport processes. The results demonstrate that water flow to the growing cells of pea stems does not limit growth.

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CHARACTERIZATION OF CALCIUM PUMPS CRITICAL FOR GRAVITROPISM

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Description of Research

In the 1980s, our understanding of the management of the root response to gravity--gravitropism--has moved from a simple and little-tested hypothesis involving one growth hormone to the realization that numerous chemical and biochemical factors are integrated in a series of steps, which are being examined at an increasingly cellular and subcellular level. It is clear that several tissues coordinate the growth of roots towards the gravitatational force, these being: (a) the root cap, where the gravity vector is perceived; (b) the elongation zone, where differential growth results in curvature, and (c) the meristem, which links these two regions.

Several hormones and chemical messengers are presently being given serious consideration in the gravitropic response. These include indole acetic acid (IAA), abscisic acid, calcium, hydrogen ions (acid), and, for some plant species, photoreceptors. Most of these factors have been implicated in the gravitropic functions of both the root cap and the elongation zone. This research has focused on calcium and IAA. Since the functions of the root cap and the elongation zone are different, and since they are separated by the meristem, the effects of calcium and IAA as well as the flux mechanisms are likely to be different and independent of each other. In the elongation zone, calcium is found to be asymmetrically distributed during gravistimulation whereas exogenous application of calcium is ineffective in altering the gravitropic response. In the root cap, however, there is no direct evidence for calcium asymmetry during gravistimulation, but application of calcium or agents removing calcium can alter the gravitropic response. The net transport of applied calcium is directed across root caps but not the elongation zone.

The growth hormone IAA is linked to calcium by evidence that (a) in shoot tissue, the hormone and calcium transport systems operate together, moving the substances in opposite directions; and (b) in roots, the IAA transport inhibitor NPA also inhibits the directed transport of calcium across the root cap and prevents gravitropism.

It has been the directive of this laboratory to begin to characterize calcium movement in the three tissues listed above and to determine the relationship between this ion and the major growth hormone, IAA. During the past year, the target questions have concerned the manner in which calcium is managed within the

different sequential tissues involved in root gravitropism. This information is necessary to ascertain the different roles of calcium in gravitropism. (a) What are the kinetics of calcium uptake into the various gravitropic-sensitive tissues? How do these kinetics vary? (b) What are the kinetics of calcium efflux from these tissues, and how do they vary? (c) Is there a relationship between calcium movement and the acid gradient along the root? (d) Do IAA transport inhibitors affect these kinetics? (e) Do inhibitors of calmodulin, a protein known to function in calcium transport, affect the kinetics? (f) What is the distribution of naturally occurring calcium along the root, and does it reflect the projected roles for calcium in gravitropism?

Accomplishments

The significant and promising findings are:

(1) Uptake of calcium in all root tissues is principally by diffusion. An active component exists, however.

(2) Root cap cells take up calcium twice as rapidly as tissue from the root proper, with the tissue immediately adjacent to the root cap, the meristem, having the slowest uptake rate.

(3) Calcium uptake is affected by acid differentially in the root cap and the elongation zone.

(4) Sodium alters the uptake of calcium into root caps.

(5) Uptake and efflux of calcium is affected by the IAA transport inhibitor, NPA, but not by calmodulin inhibitors, chlorpromazine and W7. Calcium fluxes out of root cap tissue two to four times as fast as from all other tissues.

(6) Preliminary evidence shows that naturally occurring calcium is present at highest concentrations in the root cap and the elongation zone.

Significance of the Accomplishments

These findings provide evidence that the function of calcium is likely to be different in the various tissues involved in gravitropism, and they provide a biochemical basis for comparison to biochemical calcium function in animal cells, from which additional molecular and testable hypotheses can be formulated. Findings #1, 2, 4 and 5 show that the root cap cells are more permeable to and process calcium more readily than cells of the root proper. This correlates with the yet-to-be-proven idea that calcium is critical for gravity perception and the communication of the gravity vector to the root proper. Finding #2 further suggests that calcium in the root cap is not transported significantly beyond the cap, since the adjacent root tissue, the meristem, shows a reduced uptake capacity. The results cannot rule out the possibility of calcium transport through cytoplasmic channels, but calcium transport data strongly suggest that calcium is moved between the outside of the cell, where the concentration is highest, and the inside, where the concentration is at hormonal levels. The function of calcium, therefore, may serve as an important step in the translation of the perception of gravity into a tranmission signal to the root proper where the

graded response will occur.

Finding #3 corresponds directly to the acid gradient found along the apical root, the cap being basic compared to the more acidic elongation zone. Calcium uptake is maximized at the acid level of each tissue, which suggests that calcium movement may be coupled directly or indirectly to hydrogen ion pumping, a common situation in animal cells. Finding #4 provides another important clue to function of calcium in the root cap as distinguished from its role in the elongation zone. A portion of calcium movement in the root cap is sensitive to sodium. One mechanism for regulating calcium in animal cells is a sodium/calcium exchange which is predominant in the efflux of calcium in cardiac muscle and giant squid axons. Such an exchange system may be operating differentially in root cells in response to a change in the gravity vector. Finding #5 is very important for determining the relationship between calcium and IAA in the root cap. It shows that NPA inhibition of gravity-induced calcium movement is not likely a result of calmodulin. On the other hand, we are presently investigating whether it could be due to an enhancement of calmodulin activity. These investigations are providing the important foundation for biochemical-molecular studies to detail the specific role of calcium and the sequence of events leading to transmission of gravity perception to the site of response in the root. Finally, Finding #6 provides the first evidence, preliminary though it be, of the total calcium distribution occurring naturally in young seedling roots (these roots are responsible for the establishment of the seedling in the ground and are essential for shoot growth). The distribution pattern is similar to that of calcium uptake; calcium is concentrated in root caps and in the elongation zone. Thus the root indeed does accumulate calcium normally in those regions that are the most responsive to applied calcium, making the case stronger that calcium is a <u>real</u> factor in the root cap.

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THE ROLE OF ACID AND CALCIUM GRADIENTS IN GRAVITROPISM

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Description of Research

This research is directed toward understanding the influence of gravity on plant growth--in particular the mechanism by which roots become oriented and grow in the direction of gravity (gravitropism). It is known that the detection of gravity occurs at the tip of the root while the adjustments in growth rate occur in the growing region about 0.5 cm behind the tip. During the past couple of years we accumulated evidence that gravity-induced redistribution of calcium within the tip of the root plays a key role in linking gravity detection to the altered growth pattern which leads to reorientation of the root. We also obtained evidence that the primary cause of the altered growth pattern is gravity-induced asymmetric distribution (or activity) of the growth suppressing hormone, auxin, in the growing region of the root. The major evidence indicating calcium redistribution as a mediator of gravity effects on roots was: (a) calcium-immobilizing agents applied to the tip caused roots to become insensitive to gravity; (b) calcium gradients across the tip caused gravitropic-like curvature toward the calcium; (c) gravity caused preferential movement of applied calcium across the root tip toward the lower side. The major evidence indicating the growth hormone auxin as the ultimate cause of the differential growth that causes bending was: (a) small gradients of auxin applied to the growth zone caused root curvature similar to that induced by gravity; (b) when radioactive auxin was applied to the growth zone of a root, gravity stimulation caused the hormone to move preferentially toward the lower side; (c) gravity-induced movement of calcium and auxin was tightly linked, i.e., inhibitors of auxin movement also inhibited calcium movement.

During 1984, research on the interaction of auxin and calcium centered on the following questions: (a) How does gravity cause redistribution of calcium and how does this lead to root reorientation? (b) Roots of some plants are known not to respond to gravity if they are grown in the dark. Do such roots exhibit normal gravity-induced calcium movement and hormone movement? If not, why not? What can we learn from the timing of the development of sensitivity to gravity when these dark-grown roots are exposed to light? (c) Are the differences in sensitivity to gravity in the dark and the light due to inherent differences in the sensitivity of the roots to hormones? (d) Does stimulation by gravity cause calcium redistribution in the growing zone of the root as it does in the tip? (e) Can calcium gradients influence hormone distribution in the growing zone? (f) Does the level of calcium within cells of the root determine the

effectiveness of the growth hormone auxin? The questions dealing with the way that calcium becomes redistributed and how this might influence root orientation were addressed by studying the occurrence and distribution within roots of a small protein, calmodulin, known to mediate the action of calcium in many cases. The questions dealing with differences in the gravity response in light-grown and dark-grown roots were studied by measuring gravity-induced curvature as well as calcium and hormone movement in dark-grown and light-grown roots. The questions dealing with the relationship between calcium movement and hormone movement were studied using radioactive isotopes of calcium and hormone to trace their movements. The importance of calcium to auxin action was studied by raising seedlings in calcium-deficient media and testing the response of the roots to auxin and to gravity.

Accomplishments

The major findings from these studies are:

(1) Roots of calcium-deficient seedlings are insensitive to concentrations of hormone that strongly suppress growth in normal roots.

(2) Gravity stimulation causes calcium to move toward the lower side of the growing zone of roots.

(3) Gravity stimulation causes hormone to move across the elongation zone toward the lower side. This movement is strongly enhanced when additional calcium is applied to the lower side.

(4) Maize roots contain calmodulin and it is fourfold more concentrated in cells of the cap than in cells of the elongation zone.

(5) The calmodulin level is very low in the caps of dark-grown roots. These dark-grown roots do not show gravitropism, and gravistimulation does not cause redistribution of calcium or hormone. Upon illumination there is a parallel increase in calmodulin activity and gravity sensitivity.

(6) Roots of calcium-deficient maize seedlings show greatly retarded responses to gravity.

(7) Light-grown roots (which are gravity sensitive) are much more sensitive to growth hormone than dark-grown roots (which are gravity insensitive).

Significance of the Accomplishments

Finding #1, that calcium-deficient roots have little sensitivity to growth hormone, is especially significant because it provides a potential explanation of the manner in which a gravity-induced calcium asymmetry may lead to a growth asymmetry (root reorientation). If cells higher in calcium are more susceptible to growth suppression by the hormone, gravity-induced curvature could arise simply by movement of calcium to the lower side (see Finding #2), since this would be expected to enhance the effectiveness of the growth-suppressing hormone which is already there. Similarly, movement of calcium out of cells along the upper surface would allow maximal growth there. This interpretation is important since it provides a model to account

for differential growth independently of the establishment of a hormone gradient.

Finding #2 indicates that gravistimulation causes calcium movement toward the lower side of the elongation zone. This is very important since such a gradient could account for growth modification leading to root reorientation (see Finding #1).

Finding #3 shows that gravity stimulation can cause auxin to move to the lower side of the growth zone and that this movement is enhanced when extra calcium is present in the lower side. This is significant for two reasons: (a) It confirms earlier work showing that gravity can induce asymmetric auxin movement across the growing zone of roots. (b) It shows that the strength of gravity-induced auxin redistribution is dependent upon calcium distribution in the roots. This provides a strong possibility for linking gravity-induced calcium movement to hormone movement. This may allow us to construct a comprehensive model linking calcium/auxin movement to stimulus/response coupling in plant responses to gravity.

Findings #4 and 5 show that maize roots possess calmodulin, that it is concentrated in the tip, that it is lacking or deficient in dark-grown roots, and that its activity increases upon illumination as the roots develop the ability to respond to gravity. The findings are significant in that they indicate that calmodulin may be important to the mechanism by which gravity causes calcium movement and/or the mechanism of action of calcium gradients in controlling root reorientation.

Finding #6, that calcium-deficient roots are slow to respond to gravity, is a further indication that calcium is critical to the gravity-response mechanism in roots.

Finding #7, that dark-grown, gravity-insensitive roots are less sensitive to auxin than light-grown roots, is important since it indicates that differential sensitivity to auxin may be critical to the gravity-response mechanism (see discussion of significance of Finding #1).

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BIOCHEMICAL PROCESSES ASSOCIATED WITH ROOT GRAVITROPISM

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Description of Research

On Earth roots respond to gravity by growing downward. Research conducted in this laboratory focuses on elucidating the biochemical processes that "tell" the root the direction of gravity and hence that determine downward growth.

We now know that gravity is perceived in a specialized region of the root, the root cap. As a result of gravity perception, it is hypothesized that a signal or message is formed in the cap and then moves from the cap a distance of several mm to the region of the root where bending can take place, thereby allowing the root to orient with respect to the gravitational field.

This research has been directed toward establishing the chemical nature of this signal. In addition, we have attempted to isolate and purify the component within the root cap cells that we believe is involved with production of the signal. For our work we have used a mutant of corn in which the gravitropic bending occurs only if the roots are exposed to light. If roots are grown in the dark no bending occurs. So light removes a block in certain biochemical processes necessary for signal production and hence for the gravitropic response. Using dark- or light-grown roots we have investigated the production and distribution of substances hypothesized to act as the signal.

Accomplishments

The major findings from these studies are:

(1) Two hypothesized signals, the substances abscisic acid (ABA) and xanthoxin (Xa), are found in root caps of dark-grown roots. Little ABA is found in the bending zone of dark-grown roots.

(2) When roots are illuminated bending occurs, and ABA rapidly moves from the cap to the bending zone.

(3) Light stimulates Xa synthesis in the cap and causes a rapid increase in this substance in both the cap and in the bending zone.

(4) Root caps are rich in a pigment known as violaxanthin. Following brief illumination, violaxanthin levels decrease to 40% of that in roots maintained continuously in the dark.

(5) Amyloplasts, which are components of root cap cells, have been isolated and purified.

Significance of the Accomplishments

Finding #1, that caps of dark-grown roots are high in ABA while the bending zone is low in ABA, is significant because it suggests that if ABA is the gravitropic signal, it is normally sequestered in the cap. Further, this result suggests that gravistimulation may involve the release of ABA from the cap.

Finding #2, that light causes a release of ABA from the cap and the movement of this substance into the bending zone, is especially significant. This movement occurs rapidly and within the same time frame as the light-induced bending. This result strengthens the case for calling ABA the root cap signal.

Finding #3, that light stimulates the synthesis of the substance Xa and its movement to the bending zone, is an observation of great interest. Xa has also been suggested by some to be the signal, or part of a group of signals, moving from the cap to the bending zone. Its response as a result of light treatments supports the hypothesized involvement of Xa in some aspect of root gravitropism.

Finding #4, that violaxanthin is found in the root cap and that it disappears with light treatment, is a finding of interest because violaxanthin may be a chemical precursor to both ABA and Xa. Therefore, in roots in which light stimulates gravitropic bending it may be that light acts by causing the disappearance of violaxanthin and its conversion into one or more proposed root cap signals.

Finding #5 is a report of the isolation and purification of amyloplasts from root cap cells. This is a significant accomplishment since it now allows us to explore the physiology and biochemistry of a component of root cap cells hypothesized to act as the gravity perceptor/transducer for roots.

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MECHANISMS OF GRAVITROPIC PERCEPTION AND RESPONSE IN ETIOLATED PEAS

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Description of Research

Our primary goal is to understand how plants perceive a gravitational stimulus and then use this information to orient their organs with respect to the Earth's gravitational field. As a corollary of this research, we have also become interested in the effects of the microgravity environment of space on the development of the various organelles of the plant cell, including the presumed gravity receptors.

Numerous studies suggest that the perception of gravity in plants occurs when especially dense starch-containing bodies called amyloplasts sediment in the cell in response to the gravitational field. This sedimentation, involving close physical contact between amyloplasts and other organelles, causes numerous biochemical and biophysical changes that provide the cell with a means of reacting to the original gravitational stimulus. Clearly, detailed knowledge of the amyloplast, the only cellular body capable of sedimenting in a 1 x g (gravitational) field, is essential to a detailed understanding of gravitropism.

In recent years, this laboratory and others' have provided evidence that movement of intracellular calcium (Ca^{2+}) may be one response to amyloplast sedimentation, presumably through interaction with biological membranes that serve to compartmentalize Ca^{2+} in the cell. Some election microscopic evidence suggests, in fact, that amyloplasts themselves contain significant amounts of Ca^{2+}, but virtually nothing is known about how Ca^{2+} is transported into and out of these plastids. Since Ca^{2+} is known to act as a "second messenger" for many cellular processes, intracellular Ca^{2+} movements initiated by amyloplast sedimentation may help explain plant responses to gravity, as well as problems one might encounter under microgravity.

Intact amyloplasts have been difficult to isolate in bulk for purposes of biochemical analysis. In 1983, using dark-grown pea stems and Urografin, a new dense isolation medium, we achieved the first successful bulk preparation of gravity-sensing amyloplasts from higher plants. But those amyloplasts had leaked calcium during the isolation procedure, due to the ionic nature of the dense medium used. Therefore we turned to Nycodenz, a new high-density, non-ionic centrifugation medium, which seems to obviate leakage problems. We are now using Nycodenz-isolated plastids for the study of Ca^{2+} exchange between amyloplasts and the rest of the cell.

Accomplishments

(1) We have improved our amyloplast isolation technique to yield organelles that are not only structurally intact, but that have not leaked significant quantities of their Ca^{2+} into the isolation medium.

(2) These isolated amyloplasts take up significant amounts of radioactive calcium ($^{45}Ca^{2+}$) from the medium in which they are suspended.

(3) $^{45}Ca^{2+}$ uptake is largely dependent upon membrane-localized enzymes that hydrolyze adenosine triphosphate (ATP), an energy-rich substrate that drives many reactions in the cell. Such ATP dependence is shown by the following experimental findings:

(a) Added ATP promotes $^{45}Ca^{2+}$ uptake.
(b) Inhibitors of ATPase, such as oligomycin and DCCD, greatly inhibit $^{45}Ca^{2+}$ uptake.
(c) ATPase activity and $^{45}Ca^{2+}$ uptake are positively correlated.

(4) There is also a small passive (non ATP-dependent) influx of $^{45}Ca^{2+}$ into intact isolated amyloplasts, as shown by the fact that A23187, a calcium ionophore (which makes membranes more permeable to Ca^{2+}) increases total $^{45}Ca^{2+}$ in the amyloplast.

(5) Amyloplasts seem to be able to sequester large amounts of calcium. Such sequestration is dependent upon the availability, and presumably uptake, of phosphate ions. This may indicate Ca^{2+} sequestration as calcium phosphate.

Significance of the Accomplishments

This new method for the isolation of structurally and functionally intact amyloplasts permits us for the first time to study the functional physiology of these organelles outside the cell. We are well underway to elucidate both the active and passive pathways to Ca^{2+} uptake by amyloplasts, as well as their ultimate capacity to sequester Ca^{2+}. Future studies will seek to determine how sequestered Ca^{2+} is lost from the amyloplast.

In parallel studies, we have investigated various aspects of the metabolism of polyamines (PAs), organic cations that mimic Ca^{2+} in many aspects of cellular physiology. PAs rise significantly during stress, and such PAs may influence Ca^{2+} exchange between amyloplasts and the rest of the cell. Like Ca^{2+}, PAs are reported to act as "second messengers," activating phosphorylating enzymes (kinases) that affect the function of key enzymes in the cell.

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THE ROLE OF GRAVITY IN REGULATION OF LEAF BLADE FORM

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Description of Research

This laboratory is studying the factors that maintain the dicotyledonous leaf blade in a flat form and horizontal position. Previous research (1980-1983) indicated that the major regulatory factors are auxins and ethylene. The growth of cells in the epidermis and bundle sheath parenchyma is auxin-limited. Asymmetric growth is the likely consequence of asymmetric auxin distribution. It results in hyponastic or epinastic curvature which manifests loss of the flat planar form. The major factor influencing this auxin-produced asymmetric growth is gravity. Other modifying factors are temperature, pH, light, and treatment with auxin transport inhibitors. The position of the leaf blade is determined by the angle of the laminar pulvinus. In addition to its nyctinastic sensitivity, the pulvinus is responsive to ethylene production stimulated by high auxin concentrations or changes in orientation to gravity.

During 1984, the following questions were investigated:

(1) Is calcium involved in the gravitropic responses of the blade? To answer this question, we studied: (a) the effects of exogenous applications of calcium and the chelating agent ethylene diamine tetraacetic acid (EDTA) in various combinations with each other and with indole-3-acetic acid (IAA), applied asymmetrically or in sequence; (b) subcellular localization of mobile calcium ions in situ using phosphate-buffered aldehyde-antimonate fixatives; (c) subcellular localization of crystalline calcium oxalate; (d) various histochemical tests for calcium and subsequent study by SEM, TEM, and light microscopy; and (e) effects of lithium on blade and pulvinar nastic curvature.

(2) Can asymmetric dorsiventral auxin movement through the blade be demonstrated? To answer this quesiton, we measured the collection of ^{14}C-IAA in receiver discs from dorsal or ventral donors through leaf discs positioned normally or inverted.

Accomplishments

The major findings from these studies are:

(1) Calcium ions are localized at the tonoplast of parenchymal cells (antimonate precipitation technique). Calcium oxalate stain reveals preferential distribution in epidermal cells, vascular parenchyma, and the lower layers of bundle sheath parenchyma. Calcium oxalate crystals were observed in bundle

sheath parenchyma and at the base of ventral trichomes.

(2) Applications of calcium, lithium, or EDTA have no effect on leaf blade form or position.

(3) Radioactive auxin transport studies using the methods employed do not provide conclusive evidence of gravipositive auxin movement. Polarity is evident, but the amounts collected are not sufficient to account for the growth responses observed.

Significance of the Accomplishments

Finding #1: The asymmetric distribution of calcium in the untreated leaf blade localized calcium in the very tissues previously identified as those showing a differential growth response to IAA treatment. Cells that do not respond to IAA treatment or changes in orientation to gravity (the palisade and spongy mesophyll cells) do not show accumulation of calcium. We now propose to study the effect of auxin treatment on this calcium distribution. The very promising observations thus far suggest that since calcium distribution is related to the sites of auxin-promoted growth response, it may be involved in gravitational response in the leaf.

Finding #2: The failure of calcium or EDTA treatment across concentrations ranging from 10^{-3} to 10^{-7} M to produce or affect nastic curvatures suggests that calcium is not a limiting factor for this response. Endogenous levels revealed by histochemical studies are presumably sufficient to maintain the response.

The lack of blade or pulvinus response to lithium separates the observed leaf gravisensitive curvatures from the lithium-sensitive, ethylene-regulated, nyctinastic response. This reinforces the likelihood that a separate mechanism is involved in the regulation of blade position with respect to the gravitational vector.

Finding #3: Although basipetal auxin movement can be demonstrated, our radioactive auxin transport studies do not show a quantitatively impressive collection of auxin in ventral (lower) surface receivers from dorsal (upper) donors. However, the lower epidermis is an intact layer which ordinarily does not secrete auxin and is therefore not directly comparable to the lowest layer of cells in the cut ends of stem, root, or petiole sections usually used in studies of this kind. We have recognized this difference, but thought it necessary to attempt the comparison. We believe it will be more useful to study auxin distribution in the blade by other techniques such as immunoassay using monoclonal antibodies, which we plan for the future.

THE ROLE OF GRAVITY ON THE REPRODUCTION OF MOUSE-EAR CRESS PLANTS

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Description of Research

The shape, growth patterns, movement of water and nutrients, and many other functions of plants are affected or influenced by the direction and magnitude of the force of gravity. The plant, in order to have its stem grow upward and its roots grow downward, requires the presence of gravity. A vertical stem continues to grow upward when a plant is left undisturbed. Even when the plant is placed on its side, the stem, which is now horizontal, continues to grow. When the plant is left on its side for several minutes, however, the stem begins to bend in a region a few inches from the tip and continues to bend upward until the tip region is again in a vertical position. The rate of bending and the time interval before bending can be detected depend on the type of plant.

In our laboratory we are using an apparatus called a horizontal clinostat. This device rotates plants slowly around a horizontal axis once every 2 minutes and, in doing so, exposes the plant to gravity equally in all directions on a plane perpendicular to the horizontal stem. Any bending response of the stem to gravity at any particular time of rotation is negated by an equal bending response in the opposite direction one-half revolution later. Therefore, on a clinostat, the response of plants to gravity is negligible, and in the laboratory we can then simulate for plants the microgravity environment of a satellite.

Since gravity is obviously necessary for the normal development of plant shape and growth patterns, the question arises whether gravity is also necessary for the multi-step process of plant reproduction (that is, producing seeds). Using clinostats to answer this question, we selected the mouse-ear cress plant (Arabidopsis thaliana) for its ability to rapidly produce seeds in about 35 days after planting. This makes it possible to grow several generations continuously in clinostats in a reasonable time. In theory, the third generation of plants grown on the clinostat should not have any tissues that experienced Earth gravity in a normal way. In these studies, plants were grown continuously for four generations on clinostats. Seeds or plants were removed from the clinostats only for a short time during harvest or planting. The results measured on clinostatted plants were compared to the same generation of control plants grown in an upright position. One group of control plants were rotated around a vertical axis to determine the effects and magnitude of vibration and movement from the clinostat apparatus on plant growth. These effects were found to be insignificant. The other

control group was tested in a stationary position. These three groups formed the three lines of plants where each subsequent generation received the same gravity treatment as did its parent.

Accomplishments

The major findings on each generation of plants treated with horizontal rotation (simulated microgravity environment) are:

(1) The appearance of flowers, growth of seed pods, and maturation of seeds were delayed.

(2) The total seed weight and the number of pods produced were less, compared with controls.

(3) Seed viability as measured by germination percentage did not differ from the control plants.

(4) More roots, leaves, and stems were produced as measured by fresh and dry weight. The time when plants shift from the vegetative growth phase to the reproductive phase was delayed (see #1), and as a result more vegetative parts (roots, leaves, and stems) were produced.

(5) Multiple stems were formed on clinostatted plants as compared to normal single-stem plants.

(6) The above changes appeared in the first generation of clinostatted plants. The changes remained relatively the same over the generations tested.

(7) The fourth generation of the clinostatted line when grown upright and stationary did not differ significantly from the fourth generation of stationary upright plants.

Significance of the Accomplishments

The overall finding that clinostatting induces changes that are not artifacts but that appear to be real is especially significant and thus gives insight as to the role gravity plays in the reproductive process of plants.

Finding #1 of delayed appearance of flowers and seeds and Finding #4 of production of more root, leaf, and stem tissues in clinostatted plants indicate that gravity does have a role in plant reproduction. Ground-based laboratory and long-duration space experiments in microgravity are needed to further study these changes and elucidate possible consequences on seed production (Finding #2).

Finding #5 of development of multiple stems in clinostatted mouse-ear cress plants supports the notion that a redistribution of a plant hormone, perhaps indole acetic acid, occurs in clinostatted plants. Single stem growth is mediated in many plants by indole acetic acid and the manner in which the hormone is distributed by gravity. That a change in gravity will redistribute this hormone is well documented. The putative redistribution of the hormone by clinostatting was more than likely the cause of the multiple stem growth in the mouse-ear cress plants. A redistribution of nutrients and photosynthates may have also occurred and affected reproduction.

In the studies leading to Finding #7, the fourth generation of the clinostatted line was grown upright and stationary to test whether changes observed in the third generation were due to genetic adaptation or were induced only by the environment created by clinostatting. The finding that no significant differences appeared when fourth generation plants from clinostatted and control lines were both grown upright and stationary indicates that changes observed for the third generation were due to the clinostat or simulated microgravity environment rather than changes due to selective pressures of clinostatting on the genes. The significance of this Finding #7 is that by exposure for three generations to a simulated microgravity environment as induced by clinostatting, (a) the genes or what might be called the "master plans" were found to be stable and were unaffected, and (b) only the expression or the "product" made under the direction of the genes or "master plans" was modified or delayed while the plant was growing in the novel microgravity environment.

MECHANISM OF GRAVITY RESPONSES IN CEREAL GRASS SHOOTS

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Description of Research

This research is directed towards unraveling the mechanism by which the swollen joints (pulvini) of cereal grass shoots respond to gravistimulation (that is, the upward bending response following lodging or prostration of shoots due to the action of wind and/or rain). Work in 1983 indicated that: (a) during the gravity perception phase, the gravity signal is received by specialized organelles called starch statoliths in the pulvinus cells, and if they are absent, upward bending in prostrated shoots does not occur; (b) during the gravity transduction phase, two types of growth-promoting hormones get distributed asymmetrically--indole-3-acetic acid (IAA) and gibberellins (GAs), with more of the free active hormones accumulating in the lower sides (where cell elongation is greatest) than in the upper sides (where cell elongation is least) of the pulvini which are responding to gravistimulation; and (c) during the cell response phase, five major proteins increase in the lower halves and two in the upper halves, as compared with those in upright controls. One of those that increases significantly in the lower halves is invertase which breaks down sucrose to glucose and fructose; this increase would provide the hexose sugars required for the massive amount of cell wall synthesis that occurs during upward bending. One which increases significantly in the upper halves is cellulase which breaks down cellulose to glucose; this would loosen the cell walls so that they may fold or become corrugated under stress, as they have been shown to do when cell elongation growth is zero or very small in the upper halves and maximal in the lower halves.

Accomplishments

The major findings from these studies are as follows:

(1) Gravitropic response (upward bending) begins in pulvini of oat and barley shoots in as short a time as 15 min and as long a time as 156 min, following a circadian rhythm (24-hr clock).

(2) The upward bending response is maximal when there is 3-6 cm of plant tissue beyond the pulvinus, providing the hormones and/or weight necessary to cause maximal rate of bending.

(3) The grass shoot pulvinus can lift over 1,000 times its own weight! Its weight is 0.02 gm, and it can lift a 20 gm weight to 55^{o} in 48 hr.

(4) Young grass shoot pulvini, which have no starch

statoliths present yet, cannot respond to gravistimulation. Older pulvini, which have starch statoliths present, show full response to gravistimulation, but when the starch in these statoliths is removed by alpha-amylase treatment, the upward bending does not occur. It is restored by feeding the shoots with 0.1 M sucrose.

(5) At least two hormones are of primary significance in stimulating cell elongation during upward bending in cereal grass shoot pulvini, auxin (IAA) and gibberellins (GA_3, GA_4, GA_7, GA_{19}). As a result of gravistimulation, the free, active hormones accumulate in the lower halves, whereas the inactive hormone conjugates (of the GAs, at least) accumulate in the upper halves. Hormone asymmetry for IAA is achieved as rapidly as 5 min after corn seedling shoots are gravistimulated, and full asymmetry occurs after 15 min. Bending in these shoots begins after 1-3 min.

(6) Ethylene asymmetry also occurs after gravistimulation of oat shoots, but this is not seen until 6 hr after upward bending occurs.

(7) New proteins are synthesized in pulvini during the course of upward bending, five in the lower halves and two in the upper halves. Of importance is that one of these in the lower half is invertase and one in the upper half is cellulase. The former is important in cell wall synthesis and the latter is essential for cell wall loosening.

Significance of the Accomplishments

We now have a much better idea of the nature of the cascade of events which occurs in gravistimulated cereal grass shoots when gravity is perceived, the signal is transduced, and the cellular response occurs. The signal is perceived by starch statoliths in the pulvini, and if the statoliths are absent, no upward bending occurs. The signal is transduced by causing unequal distribution of the growth-promoting hormones, IAA and several gibberellins. This occurs very rapidly for auxin, almost simultaneously with the time that upward bending is initiated. One hormone, ethylene, long thought to regulate upward bending in gravistimulated cereal grass shoots, is now ruled out as being causal in the cell elongation response. Following transduction, the asymmetric growth response requires massive cell wall and protein synthesis. We now know that the large increase in invertase in the lower halves of upward bending pulvini provides hexose sugar for the cell wall synthesis that occurs in the rapidly elongating cells there and that the cellulase increase in the upper halves loosens the walls so they can fold or corrugate under the force of upward bending of the pulvinus.

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CELLS, EMBRYOS AND DEVELOPMENT IN SPACE/MORPHOLOGY OF PLANT CELLS IN SPACE

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Description of Research

The ultimate objective of our overall research plan has been to ascertain whether flowering plants can carry out their full growth, development, and reproduction in a near-zero or hypogravity environment. Highly responsive experimental systems are being developed at different developmental or organizational levels--i.e., free protoplasts from higher plant cells which can deposit new cellulose walls, divide, multiply, embark upon organized development, and ultimately give rise to organized plantlets; free somatic cells which by division under aseptic and heterotrophic conditions may express morphogenetic competence and form somatic embryos which can, it turn, develop into plantlets; and finally at the level of plants as they develop from seeds or pre-determined growing points.

Recent emphasis has been on devising the means whereby cytological and chromosomal stability can be assessed in cultured protoplasts and cells as they undergo cell division and further development--even all the way to whole plants. The technique of special value here has been that of karyotype analysis. Our studies on chromosome morphology of the plants we work with have advanced to the point where is is possible to establish a detailed profile of the nucleus. A karyogram may be defined as a systematized or orderly arrangement of the chromosomes of a single cell prepared with the aid of photography or by drawing. The implication is that the number and morphology of chromosomes is not only representative of the cell, but typifies or karyotypes a given individual, culture, species, etc. We have adopted the view that use of rigorously monitored karyology of cell populations which are capable of undergoing organized development, even to the extent of giving rise to entire plants, provide a good means of assessing the stability or variability of the higher plant genome as it is exposed to spaceflight. We have already described some major karyological changes in plant cells from organs developed in space.

In 1984 Hemerocallis and other monocotyledonous plants routinely maintained in the laboratory were further characterized for use as model systems in plant space biology experimentation. The study of protoplast isolation, culture, and cell regeneration, coupled with chromosome analysis, were emphasized from the perspective of the question: What are the minimum and optimal

conditions necessary to permit the daylily system, or one similar to it, to become automatable? This question was further reduced to questions such as: (a) What is the best time to subculture cells intended for protoplast preparation? (b) What are the best enzymes to use to eliminate the cell wall? (c) What is the best time period for exposure to the wall-degrading enzymes? (d) What are the best protocols for removing the osmoticum as the wall-less protoplasts undergo regeneration of their walls? (e) What are the best ways to evaluate viability and vitality without involving the usual and accepted procedure of rearing morphogenetically competent units to fully organized plants? (f) What is the best way to establish karyological status? (g) How does one handle the system to render it highly predictable under spaceflight conditions?

Accomplishments

(1) We have devised the means to prepare protoplasts which are stable to repeated manipulation; (2) Onozuka P1500 cellulase, Rhozyme hemicellulase are usable or Cellulysin and Macerase are usable provided 6mM $CaCl_2 \cdot 2H_2O$ are included in the release medium; (3) release can occur within 2 hours; (4) a flotation technique which permits the protoplasts to float to the top of a centrifuge tube has been devised; (5) viability of protoplasts is demonstrable by use of vital stain but the use of chromosome analysis directly on protoplasts now permits the elimination or "sidestepping" of a lengthy period wherein protoplasts must regenerate walls before being examined cytologically; (6) karyotype analysis can also be done on material as at (5) without growing material into plants.

The best way to handle material in space is yet to be finalized. At present, it appears that immobilization of protoplasts in alginate beads as separate entities is the most readily analyzable procedure of choice because units are kept discrete and distinct as they grow. However, for quantity tests, one will have to use liquid cultures. Unlike animal cells, they do not adhere to glass surfaces. More work is needed here.

Significance of the Accomplishments

Prior to our research there were no reports of successful isolation and culture of totipotent protoplasts from any perennial monocotyledonous plant. We have devised a convenient means to collect and repeatably expose protoplasts to different environments. Little attention had been paid to the earliest stages of protoplast culture. The literature claimed isolation of protoplasts which remain as single entities, the regeneration of new wall, first cell division, further cell divisions. Thus a "protoclone" would arise from a single protoplast. Our systems contrast with the above scheme: (a) Protoplasts from cells grown in suspension did not remain single; they grouped into aggregates within 24 hr. Protoplasts from intact organs did not aggregate. (b) Since the majority of protoplasts were aggregated, wall

regeneration occurred more or less simultaneously in a number of protoplasts rather than in any single protoplast alone. (c) Therefore, strict protoclones were not formed. (d) Plantlets from such protoplast cultures are, nevertheless, identical in karyotype to each other. The work answers the question: Can plantlets generated from tissue culture be phenotypically stable so that they accurately reflect the genotype of the mature plant from which they derive? Many reports of so-called "somaclonal" variation emphasize differences. Our work emphasizes identicality provided the culture conditions are strictly controlled. It also emphasizes that tissue culture technology, when properly used, can provide a powerful tool to study plant development in space.

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THE AMYLOPLAST AS A GRAVITY-SENSING DEVICE IN PLANTS

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Description of Research

While much attention has been directed to the processes involved in gravity perception in plants, such as the role of hormonal movement, differential growth, and processes related to growth, there is still only scattered evidence about the dynamics of the gravity-sensing processes. Unless we know what the dynamics of sensing are, we will have limited bases for judging possible mechanisms for explaining gravity-induced reactions. The experiments done in this laboratory are specifically aimed to improve our understanding of the sensing dynamics, in order to clarify the characteristics of the process, rather than to directly attempt to test any given explanation of the mechanism of gravitropism. For this reason, the experimental work has been oriented to examination of the earliest events visible in the cells of plants as a change in gravity is being sensed.

In the past year, concentrated effort has been made to describe the dynamics of sedimentation of the heaviest organelles in the gravity-sensing cells: the amyloplasts. Earlier evidence indicated that even these bodies moved with insufficient rapidity in response to a change in gravity orientation to account for the gravity-sensing component of gravitropism. In order to study these dynamics in both living and in fixed cells, comparative studies were done with fixed sections of cells at intervals of time during the gravity stimulus, and with living cells observed in a section under a video microscope. The two methods give very good agreement about the movement dynamics of the amyloplasts. The capabilities for studying dynamics have been expanded considerably by the development of a computer-based method of analysis of the location of amyloplasts in the cells.

Accomplishments

Accomplishments for the past year have included the following:

(1) It was found that in both coleoptiles and in roots, sedimentation of the amyloplasts is sufficiently rapid that substantial sedimentation has been completed within the minimum period of gravity stimulation--the presentation time.

(2) Through the use of fixed tissue sections and living sections, precise calculations of the sedimentation rates have been made; maximal velocities of 19 μm/min were obtained in the gravity-sensing cells of roots.

(3) Utilizing the video microscope, it has been found that cytoplasmic streaming has a very substantial perturbing effect on the movement of amyloplasts in response to gravity.

(4) In cooperative experiments with Dr. M. Jaffe, gravitropic stimulation has been found to be associated with the rapid deposition of an unusual cell wall component: callose.

Significance of the Accomplishments

Finding #1, that the sedimentation of amyloplast organelles in gravity-sensing cells is quite rapid, indicates that these organelles are the most likely sensors, or statoliths, of the seedlings of higher plants. Their movement in response to a change in gravity orientation is rapid enough to account for the minimal time of gravity stimulation.

The calculated sedimentation rate being 19 μm/min (Finding #2) is again consistent with the evidence of these organelles being the primary sensors of gravity responses. The rate we have found is considerably higher than the rates estimated by earlier workers.

Finding #3, that cytoplasmic streaming (the active flowing of cell sap inside a living cell) has a perturbing effect on the sedimentation of amyloplasts, suggests that such streaming may contribute to the rapidity of gravity-sensing by some plant materials. We have observed cytoplasmic streaming in all gravity-sensing tissues that we have examined, and it seems possible that the streaming activity participates in the shifts of cellular components in response to gravity.

In a different type of experiment done with Dr. M. Jaffe, the formation of callose in gravity-stimulated cells is striking evidence of changes in chemical synthetic activities in the cells of plants as they respond to gravity.

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MECHANICAL STRESS REGULATION OF PLANT GROWTH AND DEVELOPMENT

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Description of Research

The project investigates effects of mechanical stresses, particularly seismic (shaking) stress, on plant growth and development. On Earth, periodic shaking or vibration significantly inhibits the growth (gain in size) and productivity (gain in mass) of many plant species growing in an otherwise low-stress environment, such as a greenhouse or growth chamber. Outdoors, wind accomplishes much the same thing. However, seismic stress does strengthen stems and petioles, so not all effects of mechanical disturbance are negative. The logical question that arises regards potential effects of mechanical stress on plants growing in an orbiting spacecraft. The jarring vibrations attending launch would likely perturb plant growth for the first several days of a 7-day mission. Effects of the more subtle vibrations attending spacecraft maneuver, machine operations, and normal astronaut activity are unknown and will require flight experiments to obtain some answers. Two scenarios of some concern are possible: spacecraft vibration may have even greater growth-inhibiting effects on plant growth in the absence of a constant gravitational field than in its presence; vibration may be perceived by plants in space as "pulses" of gravity, which may interfere with experimental treatments assuming null gravity. Thus, the aim of this ground-based research program has been to characterize effects of periodic mechanical stresses on plant growth and development and to elucidate the physiological basis for mechanical stress inhibition of plant growth. Plants are agitated periodically on a gyratory platform shaker under various environmental conditions and then subjected to growth analyses, gas exchange measurements, or measured for structural composition or growth regulator content.

Accomplishments

Significant findings resulting from these studies are as follows:

(1) Photosynthetic CO_2 fixation is temporarily inhibited by an episode of seismic stress, and appears to be a result of transitory reduction in stomatal aperture (pore size), mainly on the underside of leaves for species such as soybean and tomato.

(2) Periodic shaking also reduces the growth of leaves, which in turn limits the amount of photosynthetic surface available to increase plant mass.

(3) Plants become increasingly responsive to mechanical stresses as they are grown at ever lower light intensities. Species tested thus far are relatively insensitive to mechanical disturbance when grown at light intensities approaching full

sunlight level ($\geq$ 2000 $\mu mol \cdot s^{-1} \cdot m^{-2}$ of photosynthetically active radiation, 400-700 nm). However, soybean plants are quite sensitive to shaking when grown at typical growth chamber levels of fluorescent and incandescent lighting (i.e., $\leq$ 375 $\mu mol \cdot sec^{-1} m^{-2}$). Please note: the highest photon flux density that has been achieved thus far in the Space Shuttle Plant Growth Unit (PGU) is about 90 $\mu mol \cdot s^{-1} \cdot m^{-2}$, a level at which plant growth is quite sensitive to seismic stress!

(4) Mechanical stress inhibits the increase in size of plant cells controlled by the plant growth hormone auxin, and also disrupts the downward polar transport of auxin from the shoot tip to the growing regions of the stem. The latter may, in turn, be caused by stess-induced production of the gaseous hormone ethylene.

(5) Mechanical disturbance of sunflower plants led to a disappearance of all detectable gibberellin-like activity (gibberellin is a plant hormone that promotes elongation of stems and enlargement of leaves) from extracts of plant parts that were disturbed the most (from flapping leaves of shaken plants, or from shoot tips of stem-rubbed plants). Disturbance of leaves and stems caused roots to produce growth-inhibitory substances which were carried up into the leafy shoot in the xylem (water and mineral-conducting tissue) transpiration stream.

(6) Seismic stress enhanced modulus of elasticity, ultimate shear strength, and the cellulose component of fiber content in stems of tomato seedlings, indicating a strengthening function.

(7) Vibration of tomato seedlings for 30 min hr^{-1} at 33 Hz with displacement of stem and leaf tissues of only millimeters leads to statistically-significant reductions in plant growth, without higher-amplitude shaking or stem-abrasive rubbing.

Significance of the Accomplishments

Inhibition of photosynthetic productivity by closing stomates or inhibiting leaf growth (Findings #1 and #2) would negate many of the reasons for growing plants in space in the first place (e.g., regenerative life support). It is, therefore, necessary to characterize what growth limitations are caused by mechanical stress and how to prevent them.

With respect to Finding #3 that plant response to mechanical stress depends upon light intensity, modification of the responsiveness of plants to mechanical stress by manipulation of the light intensity may prove useful in negating or preventing undesired mechanical stress effects on plants during flight experiments. Plant growth units with such lighting capabilities have not yet been developed.

Gaining an understanding of the role of plant hormones in mediating growth inhibition by mechanical stress (Findings #4 and #5) could lead to (chemical) ways of mimicking or negating those effects, as well as an understanding of what confounding effects mechanical perturbations may have on plant responses to gravity.

The fact that seismic stress strengthens stems and petioles (Finding #6) could lead to applications whereby programmed vibrations are used to strengthen plants growing in a hypogravity environment otherwise lacking in physical stimulation. Plants growing in such an environment may become too weak to withstand sudden perturbations (e.g., spacecraft attitude or orbital adjustment).

The fact that mild vibration applied to plants growing in protective environments can significantly inhibit plant growth, although to a lesser extent than due to shaking (Finding #7), suggests that the threshold of sensitivity for seismomorphism (plant development directed by seismic forces) is quite low under certain environmental conditions, and should be corrected for or controlled out in plant flight experiments.

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FACTORS CONTROLLING THE GRAVIBEHAVIOR OF PLANT ROOTS

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Description of the Research

The objective of this research is to elucidate the mechanisms underlying how roots respond to gravity. Our approach has been twofold: (a) to chemically and genetically dissect the factor(s) determining gravicurvature; and (b) comparing graviresponses of primary (i.e., graviresponsive) and lateral (i.e., nongraviresponsive) roots in order to distinguish events critical to gravicurvature from those merely correlated with the process.

The specific experiments we conducted were as follows: (a) We analyzed gravicurvature of primary roots of seedlings having undetectable levels of abscisic acid (ABA), a plant growth regulator believed by many to be a causal agent of root gravicurvature. The ABA deficiency was induced by using genetic mutants as well as chemical inhibitors of ABA synthesis. (b) We determined if there are any differences in the structure of the presumed graviperceptive cells and tissues, and the acid-efflux patterns associated with gravistimulation of primary (i.e., graviresponsive) as compared to lateral (i.e., nongraviresponsive) roots. Indeed, an asymmetric acid-efflux correlates positively with the onset of gravicurvature by primary roots.

Accomplishments

Our experiments have provided these findings:

(1) Primary roots having undetectable levels of ABA are strongly graviresponsive, irrespective of whether the ABA deficiency is induced by genetic or chemical means.

(2) The onset of graviresponsiveness by secondary roots correlates positively with the development of an extensive columella tissue, and not with the mere presence of presumed graviperceptive cells.

(3) Tips of nongraviresponsive lateral roots, like those of graviresponsive primary roots, produce effectors capable of inducing gravitropic-like curvature.

(4) The onset of graviresponsiveness by secondary roots correlates positively with the development of an asymmetric pattern of acid-efflux by their tips. Graviresponsiveness and the development of this asymmetric pattern of acid efflux are abolished by treating roots with inhibitors of auxin transport.

Significance of the Accomplishments

Finding #1: Abscisic acid has long been considered to be the

effector responsible for, or at least involved in, directing root gravicurvature. Our results indicate that drastic (i.e., 20-100 fold) decreases in the amount of ABA (to undetectable levels) in roots does not alter root gravicurvature, suggesting that ABA is not necessary for root gravitropism. That is, our data question the long-held belief that ABA is the effector responsible for root gravicurvature. Significantly, several other labs have reported data consistent with this conclusion since we submitted the paper describing our findings for publication.

Finding #2: Although graviperception may occur in individual cells in the root cap, the mere presence of these presumed graviperceptive cells does not ensure that a root will indeed be graviresponsive. Rather, gravicurvature appears to require an extensive columella tissue, suggesting that this extensive tissue may be necessary to form the gradient of effectors necessary to induce gravicurvature. These data formed the basis for our model to account for the differential graviresponsiveness of primary and lateral roots.

Finding #3: Consistent with our model for the differential graviresponsiveness of roots is the fact that the tips of nongraviresponsive lateral roots produce effector(s) capable of inducing gravitropic-like curvature. The lack of graviresponsiveness by lateral roots is apparently due to their inability to form a gradient of the effector(s), which we believe is due to their reduced amounts of columella tissue.

Finding #4: Tips of nongraviresponsive lateral roots are characterized by an asymmetric pattern of acid efflux. The onset of graviresponsiveness by secondary roots correlates positively with the development of an asymmetric pattern of acid efflux. These results suggest that the absence of an asymmetric acid-efflux could be a factor uncoupling graviperception from gravicurvature in nongraviresponsive lateral roots. Since auxin-transport inhibitors abolish gravicurvature and the development of acid-efflux asymmetry, these results suggest that the absence of an auxin asymmetry in lateral roots (presumably resulting from a reduced columella tissue, according to our model) is at least partially responsible for the minimal graviresponsiveness of lateral roots.

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HOW THE PEA STEM SENSES GRAVITY, FRICTION, AND FLEXURE

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Description of Research

The overall aim of the research is to clarify the mechanism of gravitropic stimulus reception by seedling shoots and the early and intermediate steps in the mediational cascade.

My model for reception is that the differential pressure across the gravitropically displaced cell, exerted on its lower plasmalemmal boundary, effects rearrangements of membrane molecules controlling gravitropic calcium channels. These rearrangements permit calicum ion to flow inward from the external solution (where it is believed present at roughly millimolar concentrations) toward the cytosol (where is is believed present at concentrations below the micromolar level). It is postulated that the complex of macromolecules that is associated with a channel includes calmodulin or a member of the calmodulin family of proteins, a transport protein (porter) which when activated binds anionic auxin from the cytosol, and a kinase for the porter. As calcium ion passes inward through the channel it encounters the calmodulin, to which most of it binds. The calcium-calmodulin complex activates the neighboring kinase which then activates the porter by phosphorylating it. In consequence the porter moves auxin out the lower surface of the cell; such action by porters of many cells in series results in a buildup of auxin in the lowermost tissue as well as a depletion in the uppermost tissue. (Somewhat incidentally, but important for testing the model, incomplete capture of calcium ion by the calmodulin permits some entry of the ion into the cytosol, where the net calcium ion concentration rises slightly.)

My model for the earliest steps of mediation is that the increased auxin on the lower side leads without sensible lag to increased extrusion of protons into the apoplast. Similarly, the decrease on the upper side results in decreased secretion. Within the voltage gradient thus established, apoplastic calcium ions are displaced: the calcium ion concentration thus builds in the uppermost cells, and declines in the lowermost cells. Because under most ordinary conditions external application of calcium to a shoot inhibits growth with essentially no lag, elongation is inhibited on the upper side and stimulated on the lower side--that is, the shoot bends upward. Shortly, the proton concentration builds enough on the lower side to result in a chemical difference: an effective increase in acidity occurs. A comparable but opposite change occurs on the upper side. Because addition of acid to the apoplast elicits elongation without lag, the pH gradient reinforces the differential elongation initially

stimulated by the calcium ion gradient. Meanwhile, the increase of apoplastic acid in the lowermost tissue encourages release of cell wall-bound calcium ions to further participate in migration down what has become an electrochemical (rather than simply an electrical) gradient, while the decrease of apoplastic acid in the uppermost tissue encourages binding of calcium ions to the cell walls. The positive feedbacks of this model would work only under asymmetric conditions, whereas symmetric conditions would lead to negative feedbacks: this could account for why (auxin-mediated) gravitropic curvature can begin within a minute or two, in spite of the reports that (auxin-mediated) straight growth begins only after 10-15 min.

In order to model response to gravitational stimulation, it has also been essential to model responses to the related mechanical stimuli of vibration, friction, and flexure. Understanding the effects of vibrations is, of course, important for experimentation in spacecraft, insofar as vibration cannot always be eliminated and vibrations have particularly dramatic effects with plants with generally reduced sensory input. A partial test and an explanation of this model has been published, and it is not described further here.

Accomplishments

Three successful tests of predictions of my model for mechanical stimulation were published this year; they have formed a basis for preliminary testing of three further predictions. Earlier work using a specially developed putative bioassay to test the prediction that opening of gravitropic calcium channels should lead to a subtle increase of cytosolic calcium is being published.

The experiments on phosphorylation are progressing, but they are not yet completed and hence it is premature to speculate about whether the model will be substantiated.

Regarding the early mediational stages, equipment has been set up to test unusual predictions of the model about conditions under which auxin-induced straight growth can begin in 2 or 3 minutes. A collaboration with Anders Johnsson has been entered to measure early voltage changes. He has built a new version of the classical capacitative electrometer that he, along with Grahm and Hertz, earlier used to measure later and larger electrical changes during gravitropism.

Regarding the receptive phase of the work, enough preliminary work has been done to obtain a grant from the NSF for systematic testing of the ideas.

NSF-funded work in collaboration with Timothy Caspar and Chris Somerville shows that a mutant with defective plastid phosphoglucomutase, incapable of making as much as 5% of the normal amount of starch, carries out gravitropic bending almost

as rapidly as controls; starch-laden amyloplast statoliths thus appear unnecessary for gravitropism. The findings of this study are consistent with this model.

Significance of the Accomplishments

Restated at a more abstract level, the accomplishments are:

(1) Formally publishing experiments described earlier in progress reports to NASA;

(2) Developing improved techniques for demonstrating phosphorylation *in vivo* in plant tissue, thus making possible further testing of the central claim of the receptive model that gravitropic auxin porter is activated by phosphorylation;

(3) Establishing a collaboration, building equipment, and running preliminary experiments for test of the mediational model;

(4) Demonstrating, with support from additional sources, the inadequacy of the classical starch-statolith theory for gravitropic reception.

I consider each of the accomplishments as an intermediate step in the testing of my hypotheses for the molecular basis of gravitropism by seedling shoots. In view of the scope of the needs and opportunities for growing plants in space environments of gravitational acceleration much lower than that under which plants have evolved in their earthly environment, and in view of the paucity of current information on the molecular mechanisms of gravity responses, I believe that the systematic formulation and evaluation of molecular models for such responses is essential to the Space Biology Program and is long overdue for the field of Plant Biology. Because gravitropism is one of the most important, and certainly one of the most conspicuous, of the numerous responses to gravity, any elucidation of the molecular mechanism may be expected to lead to rapid expansion and sophistication of our abilities to predict and manipulate the behavior of plants in space vehicles and space stations.

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MECHANISM OF SHOOT GRAVITROPISM

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Description of Research

A basic but as yet unresolved problem in plant physiology is the mechanism by which plants transduce information about the direction of gravity into an oriented direction of growth. For example, when a shoot is oriented in a horizontal position it begins to curve upward in a smooth arc after about 20-30 min. Reorientation is usually complete within 2-4 hr. This curvature response (gravitropism) ultimately derives from enhanced growth of those cells comprising the lower portion of a horizontal shoot and a retardation of cell growth near the upper surface. For the past several years, our research has centered on the factor or factors responsible for this gravity-induced asymmetric growth.

During 1981-1983 evidence obtained in this laboratory and others' suggested that three agents--auxin, hydrogen ions, and calcium ions--may participate in the initiation of asymmetric shoot growth. During 1984 we have tried to critically assess the role of each of these agents in gravitropism and determine how, if at all, they interact with one another. For these studies we utilized oat coleoptile tissue (a well-studied monocotyledon system) and sunflower hypocotyls (a standard dicotyledon system).

Accomplishments

The major findings from these studies are:

(1) High molarity neutral buffers do not prevent the lateral redistribution of ^{3}H-IAA (radioactive auxin) in a gravistimulated shoot.

(2) Morphactin treatment prevents lateral ^{3}H-IAA redistribution and gravitropism.

(3) Lateral and polar auxin transport velocity are not influenced by cell wall pH.

(4) Asymmetric application of exogenous auxin (very low concentrations) can initiate substantial curvature in an upright shoot.

(5) Enhanced cell elongation and the inhibition of shoot gravitropism by EGTA (a calcium chelator) is caused by the release of H^{+} rather than cell wall softening initiated directly by Ca^{2+} removal.

(6) Quinn II (another calcium chelator) does not stimulate cell elongation.

Significance of the Accomplishments

Finding #1, that gravitropism but not auxin redistribution is

inhibited by neutral buffers, is especially significant in determining the sequence of events leading to gravitropism. That is, since neutral buffers prevent development of an acid asymmetry, a proton asymmetry cannot be the driving force for auxin redistribution. Rather, it is likely an auxin asymmetry is first established, and this event in turn initiates asymmetric acid efflux.

Finding #2 is consistent with the above order of events. Morphactin prevents the lateral redistribution of auxin and, as expected, no acid gradient develops nor is there any gravitropic curvature in the presence of this inhibitor.

Finding #3 provides yet more evidence that auxin transport (lateral or polar) is not driven by acid gradients. In these experiments an exogenous gradient was created and the velocity of auxin transport determined. Data indicated the velocity of transport is independent of pH.

Finding #4 counters arguments that the magnitude of auxin redistribution is insufficient to cause asymmetric growth. This is clearly not the case.

Findings #5 and #6 collectively suggest that calcium ions are not directly involved in those events that lead to asymmetric cell elongation. It remains to be seen whether calcium ions are indirectly involved in shoot gravitropism and/or the perception of gravity.

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ROLE OF CALCIUM AND CALMODULIN IN CONTROLLING LIGHT-MEDIATED GRAVITROPISM

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Description of Research

Light greatly accelerates the positive gravitropic response of roots in certain plants and alters the gravitropic sensitivity of coleoptiles and stems. This is taken as strong evidence that some cellular response initiated by light is the same as, or affects, one of the gravity-induced cellular responses necessary for gravitropism. The objective of this research is to elucidate the specific fundamental processes that are altered by gravity and light during the induction of gravitropic growth in plants.

One of the key photoreceptors for light-regulated gravitropism has been identified as phytochrome. Previous results have shown that Ca^{2+} and the Ca^{2+}-binding regulatory protein, calmodulin, are important agents for transducing both the photoactivation of phytochrome and the gravitropic stimulus into growth changes in plants. During 1984, research was focused on several major questions pertinent to the proposed role of calcium and calmodulin in controlling light-modulated gravitropism: (a) Where is calmodulin localized in gravistimulated plant cells and organs? (b) Can the previously reported asymmetry of Ca^{2+} distribution in gravistimulated organs be confirmed by x-ray microprobe analysis after Ca^{2+} "fixation" with antimonate? (c) Can chemically induced localized influxes of Ca^{2+} alter plastid movements in plant cells?

Accomplishments

The major findings from these studies are:

(1) In both corn and pea, calmodulin is especially highly concentrated in root cap cells, the site of stimulus perception for root gravitropism.

(2) In pea root caps, the highest concentrations of calmodulin are restricted to the columella cells, the central amyloplast-containing cells ("statocytes") which are thought to be the key gravity-sensing cells within the cap. Calmodulin distribution within the corn root cap is somewhat more diffuse.

(3) Within each central cap cell, two major organellar locales for calmodulin are the nucleus and the amyloplasts. Within the amyloplast the calmodulin is primarily in the stroma, with virtually none associated with the starch.

(4) Radiometric methods with $^{45}Ca^{2+}$ indicate that most tissue Ca^{2+} is retained in roots and coleoptiles after it is precipitated there by antimonate. Analysis of Ca^{2+} distribution in antimonate-fixed tissue by x-ray microprobe analysis reveals

that most of it is present as Ca^{2+}-antimonate precipitates.

(5) The promotion of localized influxes of calcium in Mougeotia cells by the localized application of the Ca^{2+} ionophore A23187 induces plastid movements similar to those promoted by photoactivated phytochrome.

Significance of the Accomplishments

Finding #1, that calmodulin is especially highly concentrated in root cap cells, helps explain earlier observations that calmodulin inhibitors block root and shoot gravitropism and is consistent with the hypothesis that calmodulin may be involved in the regulation of root gravitropism.

Finding #2, that even within the cap calmodulin is more highly concentrated in specialized geo-sensing cells (the centrally-located "statocytes"), further focuses attention on this regulator as being significant for gravitropic responses.

Finding #3, that calmodulin is concentrated in the stroma of amyloplasts, raises the possibility that, during the sensing phase of gravitropism, the falling amyloplast may not serve merely as a "dead-weight" indicator of gravity vector direction, but may also be redistributing regulatory functions important for the cell's gravitropic responses.

Finding #4, that antimonate efficiently fixes most cellular calcium as a Ca^{2+}-antimonate precipitate, supports the reliability of earlier studies that used antimonate as a histochemical "fixative" and concluded that a gravitropic stimulus resulted in the rapid asymmetric redistribution of Ca^{2+} in roots and shoots.

Finding #5, that a calcium ionophore can substitute for the photoactivation of phytochrome in inducing plastid rotation in Mougeotia, supports the hypothesis that phytochrome responses (including phytochrome-induced gravitropism) are mediated by Ca^{2+}. It also calls attention to the role of Ca^{2+} as regulator of plastid movements, which is pertinent for understanding the cellular factors that may regulate the rate of movement of statoliths (amyloplasts) during gravitropism.

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GRAVITROPISM IN LEAFY DICOT STEMS

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Description of Research

To understand gravitropism in stems, we need to know how gravity is perceived, what changes occur in the stem as a result of this perception (i.e., how the stimulus is transduced), and what actually happens at the level of cell growth to cause stem bending away from the Earth's center of gravity. During the past year, we have continued to investigate transduction and the mechanics of stem bending (what actually happens). Clearly, transduction cannot be properly understood until we have accurately defined the final response.

In previous work, we extended the observation that when a stem is turned to the horizontal, cells on top stop growing (elongating) or even shrink slightly, while bottom cells continue to elongate at approximately the same rate as when the stem was vertical. When the stem is mechanically restrained from bending, top cells stop elongating, although they are being stretched by growth of the bottom cells. We have asked if the halting of growth of the top cells is caused by a reorientation in the deposition of cellulose microfibrils in the cell walls. (Elongation might be halted if microfibrils were deposited parallel to the axis of the stem.) Or is the wall "tightened" in some other way, so that it resists the plastic stretching that normally accounts for stem growth? In a horizontal stem restrained from bending, pressure builds up on the bottom (caused by cell growth there) and tension builds on top. Is this pressure/tension gradient reflected in the pressures within the cells, or only in the tissues as a whole?

The classical hypothesis to account for transduction suggests that the plant growth substance auxin becomes more concentrated in the bottom of a gravitroping stem, accounting for more growth of bottom cells than top and hence for upward bending. Measured gradients in auxin concentration across gravitroping stems are either small or nonexistent, however, although the hypothesis requires that there must be virtually no auxin in top cells to account for the observed halting of growth of those cells. Instead of a gradient in auxin concentration, gravitropism might be accounted for by a gradient in sensitivity of cells to the auxin normally present in the stems. (That is, the top cells might become extremely insensitive to auxin.) We are investigating the sensitivity hypothesis.

In a more-or-less unrelated study, we are examining effects of clinostatting (slow rotation of a plant around a horizontal axis)

on flower induction in Xanthium strumarium (cocklebur), a sensitive short-day plant that is induced to flower by one night longer than about 9.3 hr. It was reported in 1962 that clinostatting inhibits induction in this species.

Accomplishments

(1) Studies with electron and polarizing light microscopes have failed to detect any reorientation of cell-wall microfibrils in top or bottom cells of a gravitroping stem. (Doctoral research of Rosemary White.)

(2) Preliminary observations indicate that walls of top cells may be somewhat thicker than those of bottom cells, and that there may be fewer microtubules (which take part in microfibril deposition; this could mean less active wall growth).

(3) Deri Thomos spent one day measuring internal cell pressures with a pressure probe. Pressures were essentially equal in cells on the top and the bottom of a restrained gravitroping castor bean stem.

(4) In one of several related experiments, hypocotyls of soybean seedlings were immersed in solutions with a range of auxin concentrations. Controls bent upward as usual, but there was some inhibition of bending at 10^{-8} M indole acetic acid (IAA) and complete inhibition with 10^{-4} M IAA. (Doctoral research of Patricia Rorabaugh.)

(5) Inhibition of bending was caused by a promotion of growth of top cells of the soybean hypocotyls by auxin (rather than inhibition of growth of bottom cells), as determined by measuring marked intervals on top and bottom.

(6) Current studies are measuring amounts of labelled IAA that penetrate the tissues.

(7) In several trials, we have been unable to repeat the reported inhibition of flowering of clinostatted Xanthium plants. Some parameters remain to be tested. (Undergraduate research project of Frank Pessler.)

Significance of the Accomplishments

Findings #1 and #2 strongly suggest that microfibril orientation is not involved in the halting of growth on top of a horizontal stem or the continued growth on the bottom. One alternative is that the ability of microfibrils to slide by one another, allowing the wall to stretch plastically, is inhibited (essentially stopped) in upper cell walls. We will now examine this alternative.

Finding #3, that internal cell pressures are similar (or identical) in top and bottom cells, supports the idea that wall "tightening" accounts for halting of growth on top (rather than decreased cell turgor pressure, an alternative).

Findings #4 and #5 could have the following interpretation: When a stem is turned to the horizontal, sensitivity of top cells to auxin is so greatly decreased that 10,000 times as much auxin is

required to promote growth (ability of walls to stretch plastically) to the same rate as before the stem was turned on its side or to the same rate as bottom cells. Thus gravitropism may occur as sensitivity to auxin changes, with greatly decreased sensitivity in top cells and little change in sensitivity of bottom cells. Observed auxin gradients or lack of them may be of little or no importance. Alternatively, it might require a 10,000-fold increase in auxin in the surrounding medium to destroy the internal auxin gradient that normally controls gravitropic stem bending (according to the traditional hypothesis). Hence, it is crucial to actually measure the auxin that penetrates top and bottom tissues of the hypocotyls (future Finding #6). If the sensitivity interpretation continues to be supported, it could change the direction of gravitropic research, a direction that has hardly wavered for almost 60 years.

Finding #7, that floral induction is not significantly inhibited by clinostatting (a possible simulation of microgravity), suggests that plant reproduction might not be adversely affected in future space experiments nor when plants are used in bioregenerative systems of spacecraft. Yet we easily inhibit flowering by applying ethephon, a source of ethylene, and ethylene is known to be produced by clinostatted plants. And we know that prolonged clinostatting does inhibit floral induction (as could prolonged microgravity in a spacecraft). Thus we continue to test parameters that might have differed between our experiments and those of Hoshizaki and Hamner in 1962. (We are in close communication with Hoshizaki.)

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ANIMAL PROJECTS

THE EFFECT OF SKELETAL UNLOADING ON BONE FORMATION

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Description of Research

The skeleton is comprised of bone cells within and around a calcified extracellular matrix which provides not only support for the body but also a major buffer to help the body regulate calcium homeostasis. The cells that form new bone (i.e., secrete the protein matrix and regulate the calcification) are called osteoblasts. The cells that absorb bone are called osteoclasts. Living bone is in a constant state of flux, called remodeling, due to the activity of these two types of bone cells. Two hormones, the vitamin D metabolite 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) and parathryoid hormone, have a major influence in regulating the activity and number of these cells. In addition, poorly understood mechanical factors in bone itself seem to regulate the activity and number of these cells. Normally the activity of the osteoblasts and osteoclasts are in balance. However, when changes in gravity occur (as in spaceflight), the mechanical forces on the bone are altered and an imbalance in bone remodeling occurs. We have developed a method of unloading the bones in the hindlimbs of rats with minimal stress to the animal. Using this model we are studying the effect of skeletal unloading on bone remodeling. We are evaluating the role of hormones as well as local mechanical factors on bone remodeling in order to understand the exact molecular and cellular mechanisms that control bone remodeling. Such knowledge should help us understand, prevent, and treat a variety of conditions that lead to osteoporosis.

Accomplishments

(1) During a 4-wk period of skeletal unloading, growth of the unloaded bones nearly ceases by the end of the first week, then returns to normal after the second week.

(2) The changes seen with skeletal unloading include a reduction in bone mass calcium content, and the ability of bone to take up radioisotopes (^{45}Ca and ^{3}H-proline), which mark mineralization of the newly formed protein matrix as well as the formation of the matrix itself. At the microscopic level, the number of osteoblasts in unloaded bones was reduced at the time bone formation was inhibited.

(3) Slightly before bone formation stops in the unloaded bones, the levels of $1,25(OH)_2D$ fall dramatically. However, when this hormone was constantly infused to prevent the fall of its concentration in the blood, the inhibitory effect of skeletal unloading on bone formation was not altered.

(4) Despite the inability of $1,25(OH)_2D$ to prevent the reduction of bone formation caused by unloading, the infusion of $1,25(OH)_2D$ actually increased total bone calcium and osteoblast number relative to the uninfused animals.

(5) Increasing the dietary content of calcium also increased the content of calcium in bone in both the unloaded and normally loaded bones. However, increasing dietary calcium decreased both bone resorption by osteoclasts and bone formation by osteoblasts.

Significance of the Accomplishments

These findings indicate that after a decrease in mechanical stress (including a reduction in gravity or muscular load) on bone, bone formation ceases permitting unopposed bone resorption. If prolonged, this could lead to subtantial bone loss and predispose the bone to fracture when normal weight-bearing is resumed. However, at least in the rat, a new steady state is reached after skeletal unloading such that bone formation and bone growth resume at a normal rate. Our findings indicate that $1,25(OH)_2D$ is involved in part since the levels of this hormone fall and rise along with cessation and recommencement of bone formation following skeletal unloading. Furthermore, infusion of $1,25(OH)_2D$ in physiological amounts increases bone formation and bone mass. However, changes in $1,25(OH)_2D$ do not entirely explain changes in bone formation with skeletal unloading since even when $1,25(OH)_2D$ levels are held constant by infusion, bone formation falls after skeletal unloading. Increasing the dietary calcium concentration also increases bone mass, but does not prevent the reduction in bone formation. Despite the inability of $1,25(OH)_2D$ and calcium to prevent the transient decrease in bone formation after skeletal unloading, they both have potential importance in increasing bone mass and making bone less susceptible to fracture after prolonged periods of skeletal unloading.

WEIGHTLESSNESS SIMULATION: PHYSIOLOGICAL CHANGES IN FAST AND SLOW MUSCLE

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Description of Research

Research is directed toward elucidating the activity-dependent characteristics of skeletal muscle that are controlled by gravitational forces. In particular, this research has focused on the functional and biochemical changes in muscle characteristics produced by simulated weightlessness as compared with the changes found in muscles deprived of neural innervation. During the 1984-1985 project period, we used a multidisciplinary approach to examine the role of the intact motor system in the maintenance of normal skeletal muscle structure and function. We have altered muscle activity by interrupting the motor system control of muscle at three levels: (a) denervation by severance of the motor nerve to the muscle, (b) spinal cord severance at the thoracic level, and (c) muscle disuse (hypokinesia) induced by lack of load-bearing of the hindlimbs while maintaining neural influences intact. We have characterized the changes in biochemical, morphological, and physiological properties of the predominantly slow soleus and predominantly fast extensor digitorum longus (EDL) muscles of rats subjected to the above conditions to alter muscle activity.

Accomplishments

Our major findings for the 1984-1985 project period are:

(1) Contractile properties of soleus and EDL from hypokinetic animals are quite different from animals with neural modifications. The speed of contraction in the soleus from hypokinetic animals becomes more like that of fast muscles, whereas, the EDL was virtually unchanged.

(2) Regulation of acetylcholinesterase, the enzyme intimately related to the control of muscle activity in response to the neurochemical transmitter, is distinctly different in soleus from hypokinetic animals as compared with denervated animals. In hypokinetic soleus the activity of this enzyme significantly increases above control levels and that of denervated soleus significantly decreases. The enzyme's activity in the hypokinetic EDL is essentially unchanged while that in the denervated EDL significantly decreases.

(3) Calcium-loading characteristics of the sarcoplasmic reticulum, an integral component of the mechanism responsible for muscle relaxation, in hypokinetic soleus increases toward that found for control EDL and significantly differs from control soleus. Again, no real differences were found between

hypokinetic and control EDL.

(4) Significant effects of denervation and reinnervation on the free radical scavenging enzymes and other mitochondrial enzymes in slow soleus and fast EDL muscles were observed. Selective modification of cuprozinc and manganosuperoxide dismutases and differential regulation of GSH-peroxidase was demonstrated in slow and fast muscles. GSH-peroxidase has a tenfold higher activity in slow muscle when compared with fast muscle. Denervation caused a significant decrease of its activity in slow but not in fast muscle.

(5) Morphologically, interruption of neural influences results in muscle atrophy with the soleus being the most affected. In hypokinetic animals only the soleus is significantly atrophied. The total muscle fiber population of hypokinetic soleus remains the same as control soleus but there is an increase in the number of fast fibers with a corresponding decrease in slow fibers.

Significance of the Accomplishments

The observed changes in electrophysiological, calcium-loading, biochemical, and morphological properties of hypokinetic soleus indicates that this normally slow muscle is changing into a fast muscle. The increase in acetylcholinesterase activity of the hypokinetic soleus to levels similar to that found in normal fast muscle is further support for this hypothesis. The fact that the EDL was mainly unaffected by the reduction of weight-bearing by the hindlimbs would indicate a differential effect of weightlessness on different muscles.

The difference in control activity and in response to denervation of free radical scavenging enzymes in slow and fast muscle may become an important marker for the determination of muscle characteristics in response to disuse-induced changes.

The predominantly slow soleus and the predominantly fast EDL both depend on intact neural influences for the maintenance of normal muscle structure and function. The soleus, which normally subserves a postural (or "antigravity") role in stance and gait, assumes some characteristics of a fast muscle, not only when the muscle is deprived of neural influences, but also when its role in weight-bearing is removed. In contrast, the EDL, which is most active in gait and does not support a sustained weight-load, is more dependent upon intact neural influence for maintenance of normal muscle structure and function. It appears that the mechanical effects induced by sustained weight-load, as in stance, are important in the soleus for maintenance of normal muscle function and structure.

The study of the physiological, biochemical, and morphological changes caused by reduced use of innervated muscle is of great importance for the prevention or attenuation of the consequences of reduced activity under conditions of weightlessness during prolonged spaceflights. Only the exact knowledge of these

processes will allow us to develop a rational approach to establish proper techniques that will prevent hypokinesia-induced changes.

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STUDIES OF INTERCELLULAR COMMUNICATION AND INTRACELLULAR RESPONSES BY BONE CELLS TO SIMULATED WEIGHTLESSNESS

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Description of Research

During the evolution of the skeletal system, the bony tissues have become responsible for two important functions: (a) as a reservoir of calcium, phosphorus, and other ions important to normal physiology, and (b) as a supportive structure which permitted upright stature, as well as a lever system for muscles to produce "work." Both these functions are responsive to activity, to work loads produced by muscle contraction, and to weight-bearing. In fact, inactivity can lead to bone loss as well as muscle atrophy, and in hypogravity or reduced weight-bearing, muscle mass, skeletal mass, and calcium loss occur rapidly. Thus, the bone cells which maintain the skeleton are extremely sensitive to the presence or absence of mechanical forces.

We are presently studying the skeletal system of animals that are placed into a non-weight-bearing posture (a system designed by E. Morey-Holton) in an attempt to mimic skeletal loss as it occurs by reduced weight-bearing or during hypogravity. We are using morphological and biochemical quantitative methods to try and determine the mechanism(s) responsible for this skeletal loss and to define what kind of "signal" is used to notify bone cells that a change in weight-bearing is occurring.

Accomplishments

In the past year the following observations about bone cells have been obtained:

(1) Bone-forming cells along the outer periphery (i.e., the periosteum) of the bone may be influenced by nerve fibers in contact with periosteal cells.

(2) Bone-forming cells along the inner surface of the shaft of bone demonstrate much less bone-forming activity than the same cells at the ends of bone.

(3) Osteocytes are cells embedded in bone and demonstrate a very low level of metabolic activity. We have demonstrated a strong 5'-nucleotidase activity associated with the cell membrane of these cells. This activity can be inhibited by binding with specific cell surface receptors. Thus, this enzyme system may be important in the transmission of a signal to indicate whether a bone is or is not being subjected to mechanical stress.

(4) Bone-forming cells demonstrated the presence of actin and other elements of a cytoskeletal system. Thus, the condition of the cytoskeleton within these cells may determine the general

metabolic activities related to bone formation.

(5) Techniques for the demonstration of calcium-stimulated enzyme activity in bone cells are being developed. Some calcium-stimulated enzymes appear to be localized at the cell membrane of certain bone cells.

Significance of the Accomplishments

Finding #1: There has been very little consideration of possible nervous influence over bone formation because there is almost no morphological evidence to indicate that nerve fibers exist near bone-forming cells. Our findings show that extremely small nerve fibers make contact with a layer of cells that in turn are in contact with bone-forming cells via special junctions. Thus, an apparent pathway exists for nervous influence over a portion of the skeletal system. This is a new concept in the field of skeletal physiology.

Finding #2: It is generally assumed that all well-differentiated bone-forming cells behave similarly. Our studies show that those cells along the compact bone of the shaft contain fewer secretory granules and fewer lysosomal bodies than similar cells at the bone ends. Thus, growth rates and metabolic activities in these two areas are significantly different and may explain differences in responses to hypogravity or mechnical force.

Finding #3: Osteocytes are buried in bone and have a much lower metabolic rate than other bone cells. It was surprising to find that these cells showed a strong and specific reaction for 5'-nucleotidase activity. This response can be inhibited by binding cell membrane receptors. This enzyme activity results in the production of a substance which could behave as a transmitter for regulating activity within the bone. This is also a new concept in the field of skeletal physiology.

Finding #4: The cytoskeletal system is a series of connecting fibers and tubules which exist within a cell to form a cytoplasmic "skeleton." This system is critical for cell movement, cell attachment to substrates, and for segregation of metabolic functions within the cell cytoplasm. The structural integrity of this system is dependent on the intracellular calcium content. It has not been previously documented that the bone-forming cells have such a cytoskeletal component. We now hope to relate this activity to the degree of weight-bearing or the influence of gravity.

Finding #5: Many of the metabolic alterations in bone cells due to hypogravity could be caused by fluctuations in calcium exchange across cell membranes. The enzymes responsive to calcium are being localized and measured, with the expectation that these specific enzymes will indicate which cells are initially altered by shifts in calcium movement.

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GROWTH AND DIFFERENTIATION OF MAMMALIAN EMBRYONIC TISSUES EXPOSED TO HYPERGRAVITY IN VIVO AND IN VITRO

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Description of Research

The purpose of this research is to determine the effects of excess gravity on development of the mammalian embryo in utero and of mammalian tissues in culture. The use of excess gravity enables us to identify systems and tissues that are sensitive to gravitational changes, and which therefore might be affected by exposure to the microgravity of space. The use of in vivo and in vitro systems allows us to distinguish any direct effects on the embryo from indirect effects resulting from changes occurring in the mother.

Accomplishments

The major findings from these studies are:

(1) The exposure of embryonic palatal shelves placed in culture was accelerated by exposure to 2.6 g.

(2) A new method of assessing in vitro palatal development was devised, using histological definitions of fusion stages to assess fusion in horizontal sections. This method, in contrast to earlier ones, allows analysis of the entire shelf edge.

(3) Interactive image analysis of embryonic limbs cultured under excess g showed that centrifuged limbs had less cartilage area than control limbs, especially in the paw region, and confirmed our initial observations of the lack of limb outgrowth in excess g.

(4) A new culture centrifuge was designed to allow comparison of right and left limbs (or palatal shelves) from a single embryo.

(5) Four-wk-old female mice exposed to excess gravities of 1.8-3.5 g for 8 wk, using a small inexpensive animal centrifuge, weighed significantly less than controls.

(6) Mice were mated after adaptation, and centrifuged animals had fewer pregnancies (11% of pairings) than did controls (29% of pairings).

(7) Centrifuged embryos were significantly smaller than controls. This difference was found to be related to g force, and not maternal weight.

Significance of the Accomplishments

Finding #1: Fusion of the embryonic palatal shelves cannot occur unless the cells of the intervening epithelia die, a genetically programmed event occurring at the same time in shelves put in

culture as it would have in the embryos. The speeding up of this process is significant because it shows that the normal timing of differentiation can be altered by altering the g force. When these results are compared with those from other excess g and μg systems, a picture emerges in which differentiation is speeded up by exposure to excess g and slowed by exposure to μg.

Finding #2: Until this method of assessing palatal fusion was developed, analyses of fusion involved the use of selected cross sections, and fusion success was scored simply as the presence or absence of fusion without regard to the stage of fusion being affected. The method developed for the gravitational studies is already being applied by us to other studies of palatal fusion involving clefting agents.

Finding #3: In order to assess limb development in centrifuged cultures, we have been using a morphogenetic scoring system, in which each element is assigned a value according to its development. Recently we have acquired a Zeiss Digital Image Analysis System and a camera lucida microscope attachment which allows us to make actual measurements of the areas of the cartilage involved. This objective analysis confirmed and expanded our more subjective measurements. ZDIAS can also provide a form factor analysis which would give us some idea of the morphometric variation, but the need for morphogenetic scoring still exists.

Finding #4: Our previous centrifuge was limited in several ways, including the number of cultures it could carry (a maximum of 60 limbs or 30 palates), its operating at only one g level at any one time, and especially by the fact that contralateral comparisons could not be made, that is, the right limbs and the left limbs from 10 embryos could be compared, but not the right and left limbs from a single embryo. Because of the great variability inherent in biological systems, and the rigorous statistics required for this type of analysis, our results often were less significant than one would have expected from simply looking at the results. The new centrifuge, which is probably the best in the world, allows comparisons of right and left limbs from single embryos, or right and left palatal shelves from the same embryo. Also, this centrifuge allows culture of 162 limbs (or palates) and can carry experiments at three different g levels at once.

Finding #5: The results using our small animal centrifuge are significant for several reasons: (a) the results parallel those found with more costly machines showing that such animal centrifuges need not be extremely expensive; (b) earlier studies often did not have a watering system and relied on potatoes as a water source--our centrifuge has a demand water supply system; and (c) the study confirms the validity of the female mouse as a model for gravitational experiments.

Finding #6: The percentage of pregnancies in control animals is

the same as in other studies. The reason for the decline in number of pregnancies in centrifuged animals may be due to effects upon the estrous cycle, or upon establishment and maintenance of pregnancies. These possibilities are currently being investigated.

Finding #7: The smaller sizes of centrifuged embryos were related to g force, not maternal weights, showing that even though mammalian embryos are immersed in amniotic fluid, they are still responsive to gravitational changes.

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THE INFLUENCE OF VARIED GRAVITO-INERTIAL FIELDS ON THE CARDIAC RESPONSE OF THE ORB-WEAVING SPIDER

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Description of Research

Prior to this NASA-sponsored work, virtually nothing was known about the Gz system of arachnids (spiders). Hypergravity (centrifugation), tilt, and vibration have been used to investigate the sensory and motor operating characteristics of the spider. Among our several findings is the demonstration that the hydrostatic system of the spider is associated with the heartbeat and is responsive to gravitational stimuli. A special effort was made during 1984 to examine stimulus parameters affecting the heart.

Accomplishments

The heartbeat of the spider is said to be neurogenic. That is, the pacemaker which stimulates the heart muscle is composed of nerve cells which lie on the surface of the heart. These cells are spontaneously active but they are also controlled by impulses arising in the brain of the spider. We have found that:

(1) Changes in the magnitude (delta Gz) and/or the direction of gravity (tilt) produce an inhibition of the heartbeat.

(2) Vibrations applied to the lyriform organ (the Gz/vibration receptor) inhibit the heartbeat of the spider.

Significance of the Accomplishments

Finding #1 confirms previous observations and tends to support the hypothesis that, in the spider, sensory and cardiac function should not be viewed independently. Further, the mediator of the Gz effects is seen to be an inhibitory control system.

During a previous period in this grant, it was demonstrated that when the lyriform organ was functionally removed the heart rate increased. This implied that an important effect of this receptor (which detects strains on the exoskeleton) was inhibitory. Consequently, one could predict that the application of vibratory forces to it would inhibit the beat of the spider heart. Indeed, in several of the spider preparations, the beat stopped for many seconds! (Finding #2)

This, and earlier work in our laboratory, supports the belief that two sensory control mechanisms influence the equilibrial/cardiac mechanism in the spider: (a) neural inhibition by afferents in the abdominal nerve, and (b) a

mechanical system which appears to reduce the stimulus input directly. With respect to the nervous mechanism, we are able to offer physiological confirmation on the function of certain abdominal nerves which innervate the heart ganglion. With respect to the mechanical inhibitory system, it is believed that this latter system is the "leg lever." The Gz/vibration receptor, the lyriform organ, is very near the fulcrum of the hypothesized lever.

PUBLICATIONS

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HOMEOSTASIS IN PRIMATES IN HYPERACCELERATION FIELDS

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Description of Research

Homeostasis is the ability of an organism to maintain its internal environment at appropriate and regulated levels, even when exposed to a variety of environmental challenges. This research program has focused on the ability of a primate, the squirrel monkey, to homeostatically regulate specific physiological variables in the presence of altered gravitational fields. Our interests have focused on the physiological systems controlling sleep, temperature regulation, circadian rhythms, feeding, and drinking. We have evaluated the response of these systems to hyperacceleration fields (+g) resulting from either acute (hours) or chronic (weeks) exposure to centrifugation on an 18-ft diameter centrifuge.

Previously, we have shown that acute daytime exposure to +g leads to a depression in body temperature that persists for at least 1 hr. This response was subsequently determined to be due, at least in part, to an increase in vasomotor heat loss. As of yet, we have not monitored heat production; however, we have determined this temperature change was not caused by a change in the arousal level of the animal. More recently, we found that a similar +g exposure during the animal's night did not result in a change in body temperature. Thus, although we have evolved on a planet with a constant gravitational field, organisms are still sensitive to changes in gravity, and this responsiveness is time-of-day dependent.

During 1984, we have continued our examination of the acute effects of +g. Further, we have also initiated studies of the primate responses to chronic acceleration. Finally, we have looked at a proposed zero-g model to evaluate and extrapolate our +g data.

Accomplishments

The major findings of these studies are:

(1) Chronic centrifugation (1.5 and 2 g) of animals exposed to 24-hr light-dark cycles results in a phase shift of the circadian rhythms of feeding and drinking. The shifts are proportional to the magnitude of the field.

(2) Animals exposed to chronic centrifugation and constant light demonstrate persisting circadian rhythms of feeding and drinking, but with longer circadian periods at 2 g than at 1 g.

(3) At 1 g, squirrel monkeys have a consolidated sleep pattern with virtually all sleep occurring at night.

(4) At 1 g, patterns of feeding, drinking, and brain temperature are synchronized with the sleep rhythm and all these rhythms persist in constant light.

(5) The suprachiasmatic nucleus of the hypothalamus is a major neural control locus for the regulation of the 24-hr rhythmicity of sleep, temperature, feeding, and drinking.

(6) When exposed to Lower Body Positive Pressure, squirrel monkeys demonstrate a decline in body temperature, without a consistent change in the level of arousal.

Significance of the Accomplishments

Findings #1 and #2 demonstrate several points regarding the influence of gravity on physiological control systems that are important for homeostasis. First, many of these systems are sensitive to changes in gravity, which infers that there is a perception of gravity. However, there is no known link between these systems and the recognized gravity receptors in mammals--the vestibular organs. Second, adaptation to changes in gravity, like other environmental variables, is a three-stage process. After an initial transient response, animals show a recovery or adaptive response to a final adapted state. The time course, as well as the magnitude of these different responses, varies across systems.

Thus, the circadian system showed a decrement in rhythmicity for the first 2 to 5 days of hypergravity. Over the following week, the rhythms recovered and the animals adapted with persisting robust rhythmicity, but with a new steady-state period.

Findings #3 and #4 provide baseline 1 g data from which we are proceeding with a portion of our current year's activity. In conjunction with Finding #5, which defines a major control center for the regulation of biological rhythmicity, we hope to begin to define how control systems receive information regarding changes in gravity and how they modify their regulated levels to a new steady state.

Finding #6 provides us with a possible technique to extrapolate our +g findings with a ground-based zero-g model. Such a technique, if verified, will allow us to begin mathematical descriptions of these functions over a full range of g. Further, since certain similarities also exist between this primate model and the rat-tail suspension model, we can also perform cross-species comparisons.

Thus, it is clear that gravity has a significant role in setting the homeostatic regulation of various physiological systems. It has been predicted, and these data verify, that alterations in gravity, either acute or chronic, can be detected and will result in changes in the steady-state levels of these systems. Time of day also plays an important role in an organism's responsiveness.

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NEURAL MECHANISMS BY WHICH GRAVITATIONAL STIMULI AND STRESS AFFECT THE SECRETION OF RENIN AND OTHER HORMONES

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Description of Research

The goal of the research is delineation of the neural pathways and transmitters that mediate the changes in the secretion of renin and the antidiuretic hormone in response to gravitational and other stimuli. Evidence from this laboratory indicates that stimulation of serotonergic neurons in the dorsal raphe nucleus of the midbrain increases renin secretion, and that these neurons project to the mediobasal hypothalamus (Karteszi, et al., Neuroendocrinology 34: 323-326, 1982). The initial goal of the present project was to determine how the message got from the hypothalamus to the renin-secreting cells in the kidneys. We have obtained solid pharmacological evidence that the pathway is sympathetic. The present goal of the research is to determine by the production of discrete lesions the parts of the raphe nuclei, hypothalamus, and brainstem that are involved in regulating renin secretion. We are investigating in rats the increase in renin produced by the serotonin-releasing drug para-chloroamphetamine (PCA), the psychological stress of immobilization, and the postural stress of tilting to the upright position.

Accomplishments

The major accomplishments in the past year include:

(1) The response to PCA previously shown by us to be abolished by lesions of the dorsal raphe nucleus and the mediobasal hypothalamus was abolished by lesions limited to the paraventricular nuclei and their immediate environs, but not by sham operations or lesions in various other parts of the hypothalamus. On the other hand, the response in Brattleboro rats, which are congenitally unable to make vasopressin in the hypothalamus, was potentiated rather than inhibited.

(2) Immobilization was shown to be a reproducible and relatively potent stimulus to renin secretion. Immmobilization was carried out by permitting rats to crawl into lucite tubes, then preventing them from crawling out of the tubes.

(3) The renin response to immobilization was found to be unaffected by lesions of the dorsal raphe nucleus, and it is normal or perhaps potentiated in Brattleboro rats, which are unable to make vasopressin in their brains. However, the response was markedly reduced or abolished by paraventricular lesions and, most recently, by knife cuts behind the paraventricular nuclei.

(4) Head-up tilting of anesthetized rats was shown to produce a reproducible increase in plasma renin activity.

Studies of the effects of hypothalamic and raphe lesions are underway. In the meantime, knife cuts behind the paraventricular nucleus also seem to inhibit the renin response.

(5) In a preliminary experiment with rats suspended in such a way that their hindlimbs did not touch the ground for 1 week, plasma renin activity and vasopressin remained at normal levels. However, for 1 day there was a decline in plasma corticosterone.

Significance of the Accomplishments

The experiments with PCA are in effect a mapping expedition designed to provide the details of the pathway from the raphe nuclei to the kidneys that is responsible for the increase in renin secretion produced by PCA. At present, the serotonergic fibers from the raphe nuclei appear to feed into a brainstem-hypothalamic mechanism that mediates renin secretion in a broader sense. Thus, it is highly significant that the response to the psychological stress of immobilization is blocked by paraventricular lesions and, most recently, by knife cuts behind the paraventricular nuclei, but is unaffected by dorsal raphe lesions. This suggests that there is a basic brainstem mechanism that is responsible for renin responses to a variety of different stimuli, and that serotonergic pathway is merely one input to this system. Furthermore, at least on the basis of the very preliminary experiment with knife cuts behind paraventricular nuclei, the response to tilting is also mediated by the hypothalamus.

There is a prominent vasopressinergic pathway passing from the hypothalamus to the medulla oblongata and it has been argued that it plays a role in the regulation of cardiovascular responses. However, in neither the PCA nor the immobilization experiments was the renin response reduced in animals in which this pathway was presumably nonfunctional because they could not make vasopressin. This suggests that some other transmitter is involved.

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EFFECTS OF HYPOGRAVITY ON SYNAPTOGENESIS IN CELL CULTURE

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Description of Research

This research is concerned with determining whether gravitational forces affect the growth and cellular organization of the developing nervous system. Since it is virtually impossible to study this problem in the highly complex brain, a model system is used. This system consists of growing nerve and muscle cells in culture and observing how simulated microgravity affects their growth, differentiation, and ability to form functional, intercellular synaptic connections (i.e., neuromuscular junctions where excitable information is transmitted from the nerve to the target muscle cells).

The impetus for this research comes from the ongoing mission of NASA to explore the possibility of prolonged spaceflights. Ultimately, the survival of humans in this microgravitational environment will be limited by the ability of terrestrial biosystems to adapt to and develop under these altered gravitational forces. Gravity is, evolutionarily, the only environmental variable which has remained constant; and since it is already known, from even relatively short spaceflights, that systemic and single-cell functions are profoundly altered on exposure to microgravity, it appears highly likely that prolonged survival in space and complex embryological developments might be significantly affected in this novel environment.

In order to examine how the relative lack of gravity might influence the development of the nervous system and its interactions with target cells (such as muscle), the clinostat rotation model has been used to simulate microgravity. Cocultures of nerve and muscle cells, obtained from Xenopus embryos, are rotated on a horizontal clinostat at speeds varying from 1 to 50 rpm to cancel the gravitational vector. The growth patterns of nerve and muscle cells were noted at various times after rotation and compared with stationary sister cultures. Also, time-lapse video micrography was performed to determine whether the ability of the nerve cell to produce processes, which make connections with the muscle, was compromised. The framework for these studies derives from the suggestion of Bornens (Biologie Cellulaire 35: 115-132, 1979) that the centriole of eukaryotic cells (excluding plant cells) acts as a gyroscopic oscillator. This property of the centriole would confer on it the role of a gravisensor. It has already been established that the centriole controls cell growth and movement and that it is the center of microtubule nucleation; therefore, it is logical to

suggest that gravitational forces are very likely to influence both growth and cell movement, possibly by interfering with the normal operation of the centriolar protein cylinders. These cell functions are, of course, especially important during general embryonic development, and are most critical for nervous system organization. It is therefore essential that we understand how these functions may be affected when organisms develop in the absence of gravity.

The first phase of the research is designed to assess the effects of clinorotation on the functional morphology of nerve and muscle cells by comparing the following parameters in clinorotated and time-matched control sister cultures using video micrography of living cells: (a) muscle cell size and shape, (b) nuclear size, (c) nucleolar number and size, (d) presence of striations, (e) number of neurons producing neurites, (f) neurite length and morphology, and (g) neurite growth rates.

Accomplishments

During the first year (1984) of this work, the following have been accomplished:

(1) Designed, assembled, and constructed a clinostat-cell culture system to study neuromuscular development under simulated microgravity.

(2) Delineated test conditions in which it was found that culture temperature was unaffected by rotation or heat conduction from the clinomotor, and that vibrations in rotated cultures are minimal and did not affect cell adhesion to the substrate.

(3) Found that cell "tumbling," with the culture substrate rotating through the horizontal plane, produces profound changes in cell behavior, whereas rotation about an axis vertical to the horizontal does not affect cells and serves as a motional and vibrational control. The speed at which the most pronounced effects were observed was 1-10 rpm.

(4) Found that muscle cell size increased, energy-source (i.e., yolk platelets) metabolism decreased, appearance of striations was delayed, the nucleus enlarged, and nucleoli often fragmented.

(5) Found that neurite growth appeared normal except that width and length of neurites was reduced and a higher frequency of varicosities were present in neurites from clinorotated cultures.

(6) Found, in preliminary studies, that spontaneous and nerve-induced aggregation of acetylcholine receptors on muscle cell surfaces was reduced and disorganized after clinorotation.

Significance of the Accomplishments

These preliminary results are consistent with a working hypothesis which states that simulated microgravity produces a retardation of cell development and maturation combined with possible alterations in neuronal connectivity resulting from abnormal axoplasmic transport of materials which may be essential

to synapse formation. The findings fit into the framework of the effects being mediated through alterations in centriolar function (e.g., the enlargement of the muscle cell nuclei, fragmentation of nucleoli), including its role in the synthesis of microtubules which are known to participate in cell growth, motility, and process formation. The delineation of the working hypothesis, concerning the role of the centriole and its appended microtubular system in the sensing of gravitational forces, leads to an independent approach to its testing. Thus, agents which are known to affect the function of these cellular organelles (e.g., cytochalasin, taxol, colchicine) should mimic the effects of microgravity produced by clinorotation. Such experiments will be undertaken in the near future.

Should these results be borne out in cells developing in real microgravitational conditions (e.g., during spaceflight), it would be prudent to suggest that embryologic development of the nervous system, which depends so heavily on the precise and intricate timing of cell migration and process formation, may be seriously affected if it were to occur in the microgravity environment of space.

THE EFFECTS OF HYPERGRAVIC FIELDS ON NEURAL PROCESSING OF SENSORY INFORMATION BY THERMOREGULATORY AND VESTIBULAR SYSTEMS IN THE RAT

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Description of Research

The neural processing of sensory information in rats placed in hypergravic fields is under study. The specific neural systems considered are mammalian temperature regulation and vestibular function. Previous studies have shown that the rat, dog, and monkey have an impaired ability to regulate their body temperature when exposed to hypergravic fields. Thus, one set of experiments is directed toward clarifying mechanisms underlying this impairment in the rat. A second set of experiments is directed toward describing, in rats, the response of the vestibular system to angular accelerations. The rat is chosen as an experimental animal in both sets of experiments in part because there are studies at Earth gravity, 1 g, that provide a basic background for further studies both at 1 g and at hypergravic fields from 1.5 to 4 g.

During this past year, research on thermoregulation and vestibular function centered on the following questions: (a) Does the impaired thermoregulatory response of rats acutely exposed to hypergravic fields reflect a resetting of the set-point for shivering and other modes of heat production? (b) During prolonged exposure to hypergravic fields does the thermoregulatory system acclimate to the hypergravic field so that body temperature is maintained during cold exposure? (c) Can noninvasive techniques be developed so that the relationship of brain temperature to vestibular-evoked responses can be shown?

Accomplishments

The major findings of this study are:

(1) The set-point for temperature regulation is lowered in rats in hypergravic fields. Rats placed in a hypergravic field of 2 g reset their thermoregulatory system to maintain their temperature several degrees below 37 ^{o}C.

(2) Adaptation to hypergravic fields (2.1 g) appears to enhance the ability of rats to cope with cold exposure during centrifugation.

(3) Techniques have been developed for recording vestibular responses to angular acceleration. As brain temperature is lowered there is a delay in successive components of the vestibular-evoked response.

Significance of the Accomplishments

Finding #1, that the set-point is lowered, is significant because it provides one further step in elucidating the nature of altered mechanisms in temperature regulation. While the nature of the impairment is complex, data are interpreted as indicating that the central nervous system continues to regulate the temperature of the rat. However, the central nervous system tends to regulate core temperature at a lower than normal level for the first few hours that the rat is exposed to a hypergravic field.

Finding #2, that adaptation protects the animal, is significant because it indicates that the central nervous system can adapt to hypergravic fields. Rats raised at 2.1 g cope well with cold exposure at 2.1 g whereas rats raised at 1 g cannot maintain their core temperature as well when challenged by a period of cold exposure at 2.1 g. This experiment shows that one neural control system can function more effectively in animals adapted in hypergravic fields, and hints that animals raised in gravitational fields other than 1 g may evolve more effective control systems (e.g., cardiovascular control systems) to cope with the altered gravitational environment.

Finding #3, that vestibular-evoked responses are modified by changes in temperature, is significant in that it not only illustrates that vestibular responses can be recorded by noninvasive means but, in addition, that this technique can be used to measure small perturbations in the system as the brain temperature changes.

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STUDIES OF THE PRENATAL AND POSTNATAL DEVELOPMENT AND FUNCTIONAL DIFFERENTIATION OF THE VESTIBULAR SYSTEM IN NORMAL AND CENTRIFUGED WISTAR RATS

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Description of Research

This research is focused upon understanding the mechanisms that underlie the response plasticity of the developing mammalian central nervous system to a variety of novel environmental experiences presenting during the perinatal (Day E18 to Day PN21) period. Such developmental adaptations may result from (a) alterations in the number of neurons being generated in the impacted system, or (b) variations in the establishment of synaptic fields or pathways. Accumulating evidence suggests that both mechanisms are applicable but that the choice of the response mechanism is determined by both the degree of environmental variation to be accommodated and the sensitivity of the affected system. Our research, focusing upon neuronal generation, has basic applications for mammalian development and maturation during null-gravity spaceflight as well as for increasing our understanding of fundamental developmental neurobiological mechanisms.

Two possible mechanisms exist to facilitate organismic responses to altered functional gravity loading by varying the numbers of neurons generated during the development of vestibular and proprioceptive systems: (a) modification in the neuronal generative period by recruitment of additional generative sites, and extending the generative interval of sites with primary responsibility for the impacted system; or (b) modulation of normal neuronal cell death patterns in either peripheral or central elements of the affected system.

During prior years of this project we have developed and analyzed timed embryonic, fetal, and early postnatal specimen series, using ^{3}H-thymidine autoradiographic techniques, to fully document the normal generative sites and birth time intervals of primary neurons comprising the vestibular periphery and central nuclei as well as the brain-stem nuclei bearing primary responsibility for processing general somatic proprioceptive input.

Our objective is to determine the effects of altered gravity loading upon the functional development of mammalian vestibular and proprioceptive systems.

Accomplishments

(1) Morphometric and timing analyses of ^{3}H-thymidine-injected, staged (E11 through PN7) normal Wistar SPF rats were concluded. Timing of neuronal generation in vestibular and cochlear neurons, under normal vivarium conditions, is strongly site-specific with a firm time onset but variable generative period length.

(2) Analyses were begun of cell death timing in vestibular and cochlear nuclei in available materials derived from normal gravity Wistar SPF specimens. Two cell death intervals were recognized: shortly after initial migration of neuroblasts from generative zones (therefore very time-specific) and around PN7. Additional specimens are required during the early postnatal period to fully define the extent and duration of the second phase of normal neuronal cell death.

(3) A comparative study of cell death parameters between normal gravity (control) and flighted specimens exposed to null gravity during E13-E18 on Cosmos 1514 was concluded. Greater than normal variability in Cosmos specimens, coupled with retardation of development during spaceflight, produced numerical differences (not statistically significant) during initial cell death period (E18) (Group 1), but was without apparent differences at PN0 time frame (Group 2) following 5 days of maternal readaptation to 1 g.

(4) A study of hypergravity (2.16 g) exposure during selected embryonic stages was begun to determine the role of hypergravity functional loading on perinatal neuronal cell death in vestibular and cochlear nuclei.

Our plans are to:

(1) Conclude all cell birth thymidine-timing studies during the perinatal (E18-PN21) period in Wistar SPF. This is presently an incomplete series in the late prenatal and postnatal period of PN7-PN21.

(2) Continue three-stage functional gravity loading during development with continuous exposure to hypergravity (2.16 g) from conception through E16d (Group 1), PN0 (Group 2), and PN28 (Group 3). All specimens will be injected with tritiated thymidine on E16. All of Group 1 will be transferred from hypergravity to 1 g at E16, half of Group 2 litter will be cross-fostered to 1 g mothers at 1 g, while all of Group 3 will remain in hypergravity to sacrifice on PN28. All groups will be sampled at birth and at PN28 for shifts in timing of neuron birth intervals and comparative cell death parameters. Analysis is in progress on first test sample of animals exposed to hypergravity through Day E16.

GRAVITY PERTURBATION AS A PROBE FOR ANALYZING PATTERN SPECIFICATION IN EARLY AMPHIBIAN EMBRYOGENESIS

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Description of Research

The main drive of this research has been to employ gravity perturbation probes such as egg inversion and horizontal clinostat rotation (gravity compensation) to study the manner in which early developmental patterns are specified. Emphasis has been placed on the role cytoplasmic rearrangements play in primary axis morphogenesis (bilateral symmetrization) in amphibian embryogenesis.

The eggs of most common laboratory amphibia (e.g., Xenopus, Ambystoma) display a distinct and uniform gravity response (egg rotation with respect to gravity after fertilization). The amphibian egg undergoes rearrangements of its radially symmetrical (along the animal vegetal axis) cytoplasm after fertilization (activation) to generate bilateral symmetry (dorsal/ventral axis). Bilateral symmetrization is sensitive to gravity orientation. This gravity sensitivity allows the experimenter to use gravity perturbation probes such as egg inversion and microgravity simulation (clinostatting) to experimentally alter the normal cytoplasmic rearrangements that accompany bilateral symmetrization.

During 1984 and early 1985 gravity orientation (tilted, inverted) and gravity compensation (horizontal clinostat rotation) probes have been used to study the cytoplasmic rearrangements (monitored by light microscopy) that accompany bilateral symmetrization during fertile Xenopus egg development.

Accomplishments

The major significant findings of these studies are:

(1) Fertile eggs develop normally under conditions that mimic microgravity conditions (gravity compensation on horizontal clinostats).

(2) The polarity of fertile eggs rotated on horizontal clinostats at 12 rpm is randomized.

(3) Simulated microgravity conditions did not disrupt the normal organization (compartmentalization) of the egg cytoplasm.

(4) Fertile eggs are quite sensitive to gravity orientation. As little as 15° tilt is sufficient to alter polarity.

(5) There is wide variation in the mobility of the cytoplasm in response to gravity inversion between fertile eggs

and between batches of eggs.

Significance of the Accomplishments

These accomplishments have relevance to the question of the mechanism by which the egg cytoplasm establishes the cytoplasmic asymmetries that are necessary for primary embryonic axis formation. We have developed a "density compartment" model for egg dorsalization based on the morphological and functional organization of the egg cytoplasm into compartments. These accomplishments test and modify the "density compartment" model.

Finding #1 eliminates gravity as a primary driving force for density compartment movement during egg dorsalization. Also, it predicts that Xenopus eggs will develop normally under the microgravity conditions of spaceflight.

Finding #2 shows that the models for dorsalization that depend on sperm penetration and sperm aster growth for the primary mechanism driving dorsalization are dispensable.

Finding #3 provides additional information on the stabilization and rearrangement of the density compartments. It also supports Finding #1, that gravity does not provide a major driving force for the stabilization and rearrangements of the density compartments. The density differences between compartments become less important in this process, and other forces such as those generated by the cytoskeleton, for example, may play a major role in stabilizing and also rearranging the density compartments.

Finding #4 shows that the mechanism for establishing dorsal/ventral polarity is under subtle control.

Finding #5 shows that variations between individual eggs as well as between batches of eggs must be taken into consideration when interpreting gravity perturbation experiments. Mechanisms for polarization must be able to accommodate those variations. This observation may explain the wide variation in the response of amphibian eggs to gravity perturbation reported in the literature.

Taken together, these significant accomplishments have allowed us to modify the "density compartment" model. The new model that emerges, the "compartment shift" model, envisions the asymmetric rearrangements of cytoplasmic compartments by mechanisms that are multiple, sequential, overlapping, and subtle.

This model is more realistic and emphasizes the complexity of the problem of understanding amphibian egg pattern specification.

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STRUCTURAL DEVELOPMENT AND GRAVITY

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Description of Research

Gravity is a major factor in determining the amount of structural support required by Earth-bound organisms. The hypothesis of this research effort is that skeletal support structure will change during spaceflight and that the degree of change will be dependent upon the growth rate of the bones and the length of exposure to flight. While preparing for flight experiments, ground-based research is concentrating on a rat model simulating some aspects of spaceflight. Those aspects of flight which are simulated are a cephalad fluid shift and unloading of the rear limbs. The animal is attached to the model with traction tape applied to the tail. The animal is in a 30° head-down position with the rear limbs elevated above the ground so that they cannot bear the weight of the rat. The forelimbs are used to move the animal throughout the caging system. This model system is being used to refine experimental protocol and details for flight experiments. Data generated by this model system will be repeated in flight experiments to determine whether mechanisms for skeletal changes during unloading with and without a gravity field are, indeed, identical.

Accomplishments

The major findings during the past year were:

(1) Touching the sides of the model cage or isometric exercises against the sides of the cage did not retard the suppression of bone formation found in unloaded limbs.

(2) Increasing or decreasing dietary calcium had no effect on the decreased bone formation during unloading the rear limbs.

(3) Control rats in the animal enclosure model aboard the Space Shuttle gained the same mass per kcal food consumed as the ground controls; however, rats in space ate 85% more food and gained 70% more weight than ground control animals.

(4) Gnotobiotic rats used for the Weber flight experiment did not develop systemic arthritis as rapidly as SPF rats.

Significance of the Accomplishments

Finding #1, that rats exposed to simulated weightlessness but not allowed to touch the Plexiglas sides of the cage did not show any further suppression of bone formation when compared with unrestricted rats, suggests that resting or flexing unloaded limbs against sides of the apparatus does not interfere with suppression of bone formation.

Finding #2: When the level of dietary calcium was changed: (a)

bone marrow area was not different between control and suspended rats, but both groups showed an inverse relationship between marrow cavity size and dietary calcium implying that bone resorption at the tibial endosteum is more responsive to metabolic factors than to load-bearing, and (b) bone formation in the suspended animals was not altered by dietary calcium, but bone formation at the tibial periosteal surface was suppressed in control rats only at the lowest level of dietary calcium (0.1% Ca, 0.3% P).

Finding #3, that flight rats eat more food and gain more weight than do ground animals, suggests that animals in space may grow faster than ground animals; these data are different from data obtained with rats flown on Soviet Cosmos flights where animals were more confined and on a restricted dietary intake.

Finding #4, that using gnotobiotic rats for flight might severely affect the outcome of a flight experiment, suggests that flight animals must be identical to those rats used in ground-based experiments prior to flight to assure that possible differences in cell cycle times will not adversely influence data to be collected.

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REGULATORY MECHANISM OF SKELETAL MUSCLE ATROPHY AND FLUID AND ELECTROLYTE SHIFTS IN THE HYPOKINETIC/HYPODYNAMIC AND ANTIORTHOSTATIC RAT

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Description of Research

This laboratory has focused on investigations of muscle disuse atrophy in response to suspension hypokinesia/hypodynamia (H/H) and on further characterization of cardiovascular and fluid/electrolyte shifts in cephalad-tilted whole-body suspended rats. Regulatory mechanisms involved in muscle atrophy have been investigated in the past year.

Skeletal muscle catabolism is postulated as one of the responses to disuse atrophy from H/H. In one series of experiments we have quantitated daily urinary excretion of 3-methylhistidine (3MH) as an index of muscle protein proteolysis. We utilized a procedure, developed in this laboratory, based on ultrafiltration and automated amino acid analysis.

Contractile properties and fatigability of skeletal muscle after H/H atrophy has been investigated. This project utilized a unique *in situ* preparation where the hindlimb muscles were stimulated with trains of electrical impulses via the sciatic nerve. Blood flow was supplied to the muscles by the intact circulatory system of the animal. This is a more physiological preparation than many other *in vitro* techniques.

In an attempt to understand why H/H suspension results in increased muscle fatigability, we have investigated several potential mechanisms. The greater rate of fatigue in disused gastrocnemius muscles may be due to a shift in the substrate used to provide energy to the contractile apparatus. We have hypothesized that disused muscles, which contain a lower concentration of mitochondrial marker enzymes and thus a lower aerobic capacity, rely on inefficient anaerobic metabolism to a greater extent for energy. During this year we completed a project which employed a unique perfused rat hindlimb technique to examine several aspects of muscle substrate utilization during resting conditions.

Another possible explanation for the decreased work capacity in disused fast-twitch muscles may be a shift in the proportion of fiber types making up these atrophied muscles. If oxidative fibers atrophy to a greater extent (i.e., reduced oxidative capacity, which we have reported) during disuse, then the more

fatigable highly glycogenolytic fibers would predominate in these muscles. Our studies on muscle fiber typing are in progress and promise to give confirming evidence to such a hypothesis.

Glucocorticoids are recognized as having a pronounced catabolic effect on skeletal muscle. We have continued investigations, started by Dr. J. Steffen while a NASA Research Associate, on glucocorticoid involvement in disuse muscle atrophy. In our recent experiments we have documented an acute increase in plasma corticosterone during initial stages (e.g., first 3 days) of H/H suspension, which was followed by a return to control level by day 7 of H/H suspension. Since tissue sensitivity to circulatory glucocorticoids may also be altered by disuse, we investigated the glucocorticoid receptor concentrations in several muscles with varying degrees of load-bearing functions.

Cardiovascular (CV) responses and fluid/electrolyte shifts during spaceflight have been attributed to a cephalad redistribution of vascular fluid. Since comparable responses can be produced in the antiorthostatic suspended rat, we investigated CV response using blood pressure parameters and a hormonal regulator of fluid and electrolyte excretions, namely, aldosterone.

Since natriuresis and diuresis have been documented in the suspended rat, we have evaluated the role of circulatory aldosterone in the regulation of these changes.

Accomplishments

(1) Disuse atrophy in the rat nonloaded hindlimbs induced a 50% increase in 3MH excretion over a 2-wk period.

(2) A major finding was that suspension-induced H/H results in a significantly faster rate of fatigue in fast-twitch gastrocnemius muscles but not in soleus muscles, which are composed of predominantly slow-twitch fibers. Despite this lower ability to maintain work capacity, atrophied muscles did not have altered speed-related contractile properties (time to peak tension, 1/2 relaxation time, peak rate of tension development, and peak rate of relaxation). In addition, disuse atrophy did not alter the fast- or slow-twitch muscle's ability to generate tension when expressed per gram of muscle.

(3) We found that glucose uptake by hindlimb muscles from 7-day suspended rats was lower than uptake rates in control rat hindlimbs. Furthermore, supraphysiological concentrations of insulin (150 μU/ml) did not stimulate an increased uptake of glucose when added to the perfusion medium.

(4) Results documented a differential effect in response to disuse on hindlimb skeletal muscle glucocorticoid receptor concentrations, with the antigravity soleus showing marked increase in receptor concentration. However, the non-load-bearing extensor digitorum longus muscle evidenced no change in glucocorticoid receptor concentrations.

(5) Suspension of rats up to 7 days produced distinct evidence of hypertension, namely, increased blood pressure (mean,

diastolic, and systolic). To a great extent these changes were most pronounced during the first 3 days, followed by a plateau at a level about 2% higher than the controls.

(6) It was found that circulatory aldosterone levels increased over 200% during 7 days of head-down tilted suspension. In contrast, orthostatic suspended rats remained comparable to the controls.

Significance of the Accompishments

Finding #1, a 50% increase in 3MH excretion induced by disuse atrophy, supports the contention that proteolyisis plays a significant role in muscle atrophy in response to H/H disuse.

Finding #2 demonstrates a decreased work capacity in disused muscles and suggests further investigation of mechanisms of adaptation.

Finding #3, a demonstration of insulin insensitivity in disused muscles, may be explained in part by our finding that there are significantly higher stores of glycogen in these disused muscles.

The significance of these investigations (Findings #1, #2, and #3) is the valuable baseline data in resting muscles from whole-body suspended rats. We are planning to expand these studies to examine substrate use during contractile activity, as well as determine the extent of total carbohydrate and lipid utilization in muscle. The use of the perfused hindlimb technology in these studies will allow us to examine whether or not decreased insulin sensitivity plays a role in limiting substrate uptake; subsequent utilizaton or shifts in substract preference may explain our finding of increased fatigability in fast-twitch muscles.

Finding #4 demonstrates an increased sensitivity of disused load-bearing skeletal muscles to catabolic humoral agents which may regulate protein synthesis.

The significance of the blood pressure studies (Findings #5 and #6) is the suggestion that there is a CV adjustment to the assummed fluid shifts during head-dowm tilting in rats. In addition, the significance of the aldosterone results is that they suggest that aldosterone alone may not be involved in the long-term regulation of the natriuretic and diuretic response to fluid shifts induced by cephalad tilting.

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HYPERGRAVITATIONAL EFFECTS ON MAMMALIAN FETAL AND NEONATAL GROWTH AND DEVELOPMENT

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Description of Research

To better understand the fundamental role of gravity in development, research is being conducted on animals to study the effects of hypergravity exposures (via chronic centrifugation) from conception to adulthood. The objectives are: (a) to determine the stages of animal development when significant changes occur, (b) to identify those structures and functions that undergo major and significant changes, (c) to establish the relationship between the changes effected and the g-intensity, through the use of graded hypergravity intensities, and (d) to determine the scaling effects of gravitational force on body size through studies on different animal species developing under hypergravity conditions.

During the past year, a series of mating, growth, and developmental studies on mice and rats were conducted under graded hypergravity intensities ranging from 1.27 g up to a maximum of 2.03 g. Studies were also initiated to adapt guinea pigs to hypergravity for mating and pre- and postnatal developmental studies.

Accomplishments

(1) Reproductive capabilities of rats showed a marked decrease with increasing hypergravity intensities while mice showed virtually no difference from 1 g controls.

(2) Body masses of newborn rats were slightly smaller than controls under the higher hypergravity intensities used but were the same at other hypergravity intensities. Similar results were found in mice.

(3) Postnatal growth rates of both mice and rats were decreased only at the highest gravity intensities and were essentially unchanged from controls at the other hypergravity intensities used.

(4) The maximum body size attained under hypergravity by sexually mature male and female mice were the same as that reached by normal gravity controls. Male rats showed a smaller maximum body size at maturity only at the highest gravity intensities, while the females showed considerably less difference from corresponsing 1 g controls.

(5) Fetal growth rates in hypergravity pregnant rats were not decreased from control rates; 22-day-old fetuses were actually found to be larger.

(6) The relative lung size of 22-day-old fetuses under

hypergravity was found to be significantly reduced, the only organ found to show this type of change.

(7) Organ/body mass ratios of both male and female rats conceived and reared under graded hypergravity at 9 wk of age were unchanged from normal gravity controls, except for the amount of abdominal fat (females) and the plantaris muscle (males and females).

Significance of the Accomplishments

Results from these studies indicate and strongly support the view that the responsiveness and the degree of change effected in the developing animal to hypergravity depend primarily on the body size of the animal. Major and substantial changes are seen in both mice and rats after birth and not during the gestation period. This view is further supported by the smaller effects produced by the same hypergravity intensities in mice, compared with rats. This, we anticipate, will be substantiated further in our forthcoming guinea pig studies.

The finding that the lung is the only organ to be significantly reduced in the developing rat fetus is interesting and surprising. This decrease in lung size may be one of the major contributory factors in the high mortality rates found in rats born under 2.03 g, reflecting a decrease in respiratory functional capacity.

Although more studies must be conducted, the results to date suggest that for developmental studies conducted in space, the selection of the animal species will be crucial with respect to determining the significant effects of microgravity on mammalian animal development. According to our experimental findings, the mouse would not be a first choice for such studies.

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GRAVITY, BODY MASS AND COMPOSITION, AND METABOLIC RATE

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Description of Research

The purpose of the research is to examine the influence of gravity on body composition and the metabolic energy requirements of terrestrial mammals. One approach we have taken is to measure oxygen consumption rate and body composition of a large series of laboratory animals of different ages and both sexes, representing five species: mouse, hamster, rat, guinea pig, and rabbit. The series provides a 100-fold range of body size, from 0.05 to 5 kg body mass, thus permitting analysis of the data for scale relationships as a function of body mass. Another approach has been to subject groups of animals of the different species to altered gravitational loading for several weeks, and then examine the effect on body composition and metabolic energy expenditure. Finally, we have examined rats after 18.5 days of weightlessness in the 1979 Cosmos 1129 flight.

Accomplishments

The major findings from these studies during the past year may be summarized as follows:

(1) Based on measurements of the creatine content of the body, we have been able to estimate the skeletal muscle mass of the body in our large series of five species of animals under normal gravity conditions. We found that the percentage of the fat-free body representing skeletal muscle, which we have termed the muscularity of the animal, exhibits no sex differences, but large age and species differences.

(2) All five species display low muscularity at 1 month of age, highest muscularity at 2-3 months of age, and a decline in muscularity out to 2 years of age.

(3) The smallest species, the mouse, displays the highest muscularity, followed by the rat, rabbit, and hamster, with the guinea pig lowest.

(4) Chronic centrifugation at 2 g for 6 weeks results in a marked reduction by 30-40% in the body fat stores in all the species studied.

(5) Total body calcium and phosphorus contents are significantly greater at 2 g compared with normal gravity.

(6) Total body potassium, nitrogen, and creatine contents are not demonstrably different at 2 g compared with normal gravity.

Significance of the Accomplishments

Based on our measurements of body creatine content under normal

gravity conditions, it is evident that among these small mammals there is no indication of scaling of muscularity to body size, despite the 100-fold difference in body mass represented. Thus, it appears that in this size range of mammals mechanical loading by Earth gravity has not been as important a natural selection factor for muscularity as other requirements on the animal. For example, there is a close correspondence between the attainment of sexual maturity and the peak in muscularity, which may indicate a primary value of optimal mobility for species survival.

The observation that the proportion of the fat-free body mass represented by the skeletal musculature reaches a pronounced peak value at age 2-3 months carries the implication that the fraction of the fat-free body represented by other components must increase complementarily in older animals. We suggest that, in all likelihood, it is the supporting components of the body, the skeleton and connective tissue, that increase as muscularity diminishes.

The striking reduction in body fat content by one-third as a result of augmenting gravitational loading, seen in all the species examined, has been observed previously in some of these same species by other investigators. The uniformity of the finding strongly implies a specific effect of increased loading on fat metabolism, the nature of which is unknown at present and is worthy of further exploration.

The increases in body calcium and phosphorus contents clearly indicate an increase of about 15% in the bone mineral mass, and hence skeletal mass, of the body when loading is doubled. This is the exact opposite of what was observed in rats after 18.5 days of weightlessness in the Cosmos 1129 flight, in which they lost about 15% of their bone mineral mass. The implication here is that skeletal mass of the body seems to respond linearly to gravitational loading over the range 0-2 g. It has been strongly suspected that other physiological functions such as the regulation of metabolic energy expenditure and of blood volume respond linearly to gravitational loading, but the bone mineral response is the first to be demonstrated experimentally in the same species from 0 g to 2 g.

In contrast, the absence of change in body potassium, nitrogen, and creatine in the chronically centrifuged animals strongly implies no increase in the muscle mass of the body at 2 g. This has also been observed previously in the rat by other investigators. While it has been shown by others that the mass of antigravity muscles such as the soleus and the gastrocnemius is reduced in the rat after 2-3 weeks of weightlessness, it was also evident from our body potassium, nitrogen, and creatine data from Cosmos 1129 that the overall muscle mass of the body was not demonstrably diminished. Hence, it appears that while the total skeletal mass of the body responds linearly to gravitational loading from 0 g to 2 g, the response of the total muscle mass of

the body is far more limited.

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BONE CELL KINETICS OF SIMULATED WEIGHTLESSNESS

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Description of Research

The vertebrate skeleton has a genetic predisposition for the distribution, shape, and mimimum mass of individual bones. Environmental factors (i.e., loading of locomotion and antigravity posturing) superimpose a complex, nonlinear influence on skeletal mass and distribution. Weightlessness of spaceflight and simulated weightlessness (30° head-down suspension of a rat) are unique experimental conditions for probing the gravity dependence of skeletal adaptation. The present cell kinetics research utilizes DNA labeling (^{3}H-thymidine), mitotic activity, and nuclear size as indices of the proliferation and differentiation aspects of osteoblast histogenesis (production of bone-forming cells). The central thrust of these studies is to determine the relative influence of gravity, loading, and physiological stress on osteoblast histogenesis in a weight-bearing bone (tibia or ulna) and a non-weight-bearing bone (maxilla).

Previous research (1982-1983) in periodontal ligament (PDL), the osteogenic interface between tooth and bone, revealed three kinetically and/or morphometrically distinguishable cell types in the osteoblast histogenesis sequence: (a) self-perpetuating, less differentiated precursor cells (A type), (b) committed osteoprogenitor cells (A type), and (c) preosteoblasts (C/D cells). The rate-limiting step in differentiation of an osteoblast (bone-forming cell) is an increase in nuclear volume, immediately prior to the last proliferation cycle (C/D cells) in the sequence. This morphological manifestation of change in genomic expression (differentiation) has proven to be an effective tool for assessing inhibition of bone formation during spaceflight. Although preosteoblast numbers are markedly suppressed, suggesting a block in osteoblast differentiation, the relative influence of actual weightlessness and/or the physiological stress of the flight itself is not established.

Studies in 1984 focused on defining the cellular compartments and timing of the proliferation and differentiation steps in the histogenesis sequence for producing osteoblasts. The following principal questions were addressed: (a) What are the arrest points in the osteoblast histogenesis sequence? (b) What is the timing of each step and the total elapsed time for the entire sequence? (c) Is there a circadian influence? (d) Is the nuclear volume index, originally derived in PDL, applicable to other bones? (e) Can the measurement of nuclear size be

automated? (f) Does ground-based simulated weightlessness influence osteoblast differentiation? Experiments were: (a) cell kinetic analysis of physiological bone formation of suspended (14 days) and control rats, and (b) rats sacrificed at hourly intervals over one complete 24-hr cycle (12-hr dark, 12-hr light).

Accomplishments

(1) When osteoblast production in PDL is inactive, such as along resorbing bone surfaces, there are at least four arrest points for cells in the osteogenic sequence: A, A, C, and D cells. The first three are predominantly in pre-DNA synthesis (G_1) block, while the latter is in post-DNA synthesis (G_2) block.

(2) Since preosteoblasts synthesize DNA during the light and divide in the dark, while their less differentiated predecessors have an opposite rhythm, the total elapsed time for osteoblast histogenesis under physiological conditions is 60 hr (five alternating dark/light cycles of 12 hr each).

(3) Increase in nuclear size to form a preosteoblast occurs during a 19-hr G_1 phase.

(4) Preosteoblast formation is inhibited by physiological stress in PDL and growth plate of long bones.

(5) Simulated weightlessness per se may have no direct influence on preosteoblast differentiation because early versions of the rat suspension model introduced significant physiological stress.

(6) A computerized nuclear morphometric method is more rapid and produces less eyestrain than direct microscopy.

Significance of the Accomplishments

Finding #1, that there are three G_1 and one G_2 arrest points in the histogenesis sequence, answered an important problem in osteoblast genesis, namely, the differential roles of C and D cells. It is now clear the former are G_1 phase while the latter are G_2 phase preosteoblasts. This was the missing piece of the puzzle that will now allow mathematical modeling of the osteogenic reaction to determine if all the major steps of osteoblast histogenesis are known.

Finding #2, that 60 hr is the approximate elasped time for the entire sequence, is the key piece of evidence for determining that the upward shift in nuclear volume occurs during a 19-hr G_1 phase (Finding #3). This calculation is possible because the intervals of the other cell cycle events were previously determined.

Finding #4, that preosteoblast differentiation is inhibited by physiological stress, is a particularly important result because all spaceflight experiments to date have been stressful to some degree. The inhibition of bone formation previously attributed to weightlessness is probably at least partially due to stress (Finding #5). In future flight and simulated weightlessness

experiments, it is important to control for physiological stress.

Finding #6, of a computerized method for measuring nuclear volume, is a step in the direction of simplifying a difficult technical task. At present this automated method is about 10% less accurate because recognition of cells not properly oriented in the plane of sectioning is not as precise. With higher resolution television microscopy, this problem can probably be diminished.

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MAMMALIAN GRAVITY RECEPTORS: STRUCTURE AND METABOLISM

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Description of Research

The long-term goal of this research is to elucidate calcium metabolism in gravity receptors. In mammals, the gravity receptors consist of two paired structures, the saccular and utricular maculas. These are located in the vestibular portion of the inner ear. "Gravity" receptors really are sensitive accelerometers which detect the direction and rate of linear acceleration (or deceleration), whether it is translational or gravitational in origin.

The investigation began with studies of otoconia, the particles of calcium carbonate that add mass to the suprastructure (otoconial membrane) of the maculas to increase the sensitivity of the underlying sensory regions (maculas) to inertial force. Early research on otoconial development showed that fetal otoconia have a highly ordered organic matrix that includes a central core. The core serves as a template of sorts around which further matrix becomes organized to produce the final otoconial configuration. Deposition of organic matrix and of calcium carbonate appears to be nearly simultaneous. This research led to questioning of the "single crystal" nature of otoconia. Later our ultrahigh resolution electron microscopy of otoconial fragments proved that they were highly ordered mosaics of microcrystallites and not single crystals. This work led directly to our more recent efforts to learn the biochemical nature of the organic material that organizes the otoconial crystallites into the configuration of a crystal of calcite in birds and mammals, or aragonite in other classes, and of vaterite (an unstable form of calcium carbonate) in certain fishes. We have sought to answer the question whether the matrix material is a glycoprotein, a proteoglycan, or some combination of both. (The two differ functionally: glycoproteins figure in calcium sequestering in other systems, proteoglycans capture water.) We also wish to learn whether small differences in amino acids determine the mineral phase laid down. Further questions are whether the organic material helps regulate calcium concentration of the endolymph, the fluid around the crystals and around the tops of the underlying sensory cells (hair cells); and whether gravity plays a role in determining the mass of mineral laid down. We are using high performance liquid chromatography (HPLC), histochemical methods involving formic acid decalcification and staining with Safranin-O, and 2-dimensional gel separations in this work.

Our ultrastructural studies of the receptor areas and their

sensory hair cells are related to the question of possible otoconial modulation of calcium in the system. This is because we have morphological evidence that one part of the hair cell that extends under the otoconial complexes may move constantly in whiplike fashion. This part is called a "kinocilium." We have also found musclelike structures at the tops of the hair cells and extending down their sides that we have called striated organelles. There are, in addition, numerous narrow channels (smooth endoplasmic reticulum) strategically located below the surfaces of the hair cells and across the nerve terminals (calyces) that enclose some of them. Such channels could figure prominently in hair cell responses and in transmission of nerve signals. All these structures would be modified functionally by calcium and may figure in adaptation to changing gravitational environments. Thus, our most recent work involves attempts to localize calcium within the sensory cells and their nerve terminals.

Accomplishments

The major recent findings are:

(1) The organic matrix of mammalian (rat) otoconia contains high concentrations of acidic amino acids.

(2) The material also contains very small quantities of proline and hydroxyproline.

(3) Analysis for neutral and amino sugars by HPLC methods revealed the presence of large quantities of galactose and small amounts of mannose, glucosamine, and galactosamine, which is compatible with the presence of glycoprotein.

(4) One protein separated out by gel electrophoresis had the molecular weight of calmodulin, typically found only inside cells. Radioimmunoassay proved there is no calmodulin in otoconia.

(5) HPLC analysis has thus far failed to reveal the presence of gamma carboxyglutamic acid (GLA), implicated in bone mineralization.

(6) Decalcification with formic acid of fixed otoconia demonstrated that otoconia of adult rats contain the same, highly ordered organic material as is present in fetuses, including a central core.

(7) Less complete decalcification demonstrated unequivocally that otoconia are covered by a thin coating of organic material.

(8) Our ultrastructural work on receptor cells has revealed a new pattern of innervation, heretofore unknown, whereby type I and type II cells are clustered into groups by sharing nerves in common.

(9) The kinocilia of the cells of a cluster are not all pointing in the same direction, which means the kinocilia are detecting different directions of inertial force.

(10) A system of nerve terminals for modulating sensory cell activity within the maculas has been discovered.

(11) Systems of channels across nerve terminals (calyces) around one kind of hair cell (type I) in the maculas have been

described.

Significance of the Accomplishments

Findings #1 and #2 indicate that the organic matrix of otoconia will be similar in many ways to that of shells and fish gravity receptor minerals (called otoliths). This is exciting, because one of the first mineralized materials to appear in the evolution of living organisms was the mineral over gravity receptors (in invertebrates). This fact alone suggests that a critical mass of mineral may be required in a graviceptor for its proper functioning in a given gravitational field.

Finding #3 suggests that glycoproteins will figure prominently in mineral seeding, growth, and maturation in otoconia just as they are implicated in other kinds of biologic mineralization (bones, teeth, shells).

Findings #4 and #5 are beginning to rule out certain substances as important in otoconial crystallization processes. The potential presence of proteoglycans, an entirely different combination of sugars and protein, cannot be ruled out. We are currently testing for them by histochemical methods.

Application of formic acid demineralization in the course of this work led to the discovery of the internal, organic matrix of adult otoconia (Finding #6). This means that the organic material does not diminish during maturation of the crystalline form, as occurs in some other mineralized structures such as teeth. The presence of such organic material lends further support to the notion that the crystals can deform under stress and may be piezoelectric (capable of altering the electric field around them as they are stressed). This would mean that otoconia may change (transduce) applied inertial force into electrical activity which could then tell the sensory cells the direction and rate of acceleration very simply, by altering the electric field. This new theory of transduction is amenable to testing both on Earth and in weightlessness.

Finding #7 means that otoconia could, indeed, take up and release calcium, as our earlier work demonstrated experimentally. One problem of interpretation of this earlier research was whether organic material was located at the surfaces and could then account for the rapidity of calcium uptake, or if the surfaces were mineralized.

Findings #8 through #11 are entirely de novo observations, which mean that processing of acceleratory information begins peripherally in the gravity-sensing system of higher vertebrates. This brings the gravity receptors into line with another specialized sense organ, the retina, in which processing of visual information has been shown to occur.

PUBLICATIONS

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Ross, M.D. and Donovan, K. Gravity Receptors: An Ultrastructural Basis for Peripheral Sensory Processing. Physiologist 27(6, Suppl.): S85-S86, 1984.

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Ross, M.D. and Pote, K.G. Some Properties of Otoconia. Philosophical Transactions of the Royal Society of London, Series B 304: 445-452, 1984.

Ross, M.D., Rogers, C., and Donovan, K. Further Evidence for Peripheral Sensory Processing in Mammalian Gravity Receptors: The Ultrastructural Organization of the Saccular Macula in the Rat (Abstract). Association for Research in Otolaryngology, 1985.

EFFECTS OF WEIGHTLESSNESS ON AURELIA EPHYRA DIFFERENTIATION AND STATOLITH SYNTHESIS

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Description of Research

The simple, relatively inexpensive Aurelia Metamorphosis Test System is being used to determine the effects of weightlessness (as caused by microgravity of spaceflight or parabolic flight) or simulated weightlessness (as caused by horizontal clinostat rotation) on the normal metamorphosis of the tiny immature jellyfish ephyrae, including their gravity receptor structures (rhopalia) and their orienting behavior. During the short-term metamorphosis period of 5-6 days at 27°C, ephyrae form new structures which are required for their swimming, pulsing, and orientation. These structures include striated muscles, a new nerve net, and gravity receptor structures. The graviceptor structures (which are also found in the inner ear of humans) include statoliths (mineral crystals resembling otoliths of humans), hair cells, and sensory neurons. Our research this year focused on the graviceptor structures and on behavioral responses of ephyrae during parabolic flight.

Clinostat research: A series of clinostat rotation experiments were done to determine whether horizontal clinostat rotation (which simulates weightlessness) causes a modification of the number of statoliths formed during graviceptor formation in ephyrae. In order to do these experiments, it was necessary to devise a method for immobilizing the metamorphosing polyps which develop into ephyrae. After considerable effort, we found that the polyps could be impaled on tiny cactus spines which are embedded in small plastic capsules. The capsules are inserted into a tube which is attached to the shaft of the clinostat. Three clinostat experiments were done wherein the metamorphosing organisms were rotated at 1/4 rpm for 6-7 days at 27°C.

Parabolic flights: In order to determine whether short-term exposure to 0 g causes visible changes in ephyrae behavior, ephyrae were flown in parabolic flights by Dr. C. Oman, MIT, using NASA's KC 135 airplane. The ephyrae were observed and videotaped by Dr. Oman. They were grown in our laboratory and then transported to Houston.

Accomplishments

(1) Horizontal clinostat rotation at 1/4 rpm of developing ephyrae caused the synthesis of statistically significantly fewer statoliths in the graviceptor structures than were found in vertically rotated or stationary controls.

(2) Parabolic flight studies of the swimming, pulsing, normal ephyrae revealed that ephyrae are behaviorally very gravisensitive, since the ephyrae stopped pulsing at 0 g and then turned and accelerated pulsing while swimming upward (tracking g) as the g force increased to 2 g.

Significance of the Accomplishments

These accomplishments demonstrate that developing and mature ephyrae are sensitive to weightlessness. The organisms which developed on the clinostat received a stimulus which caused them to synthesize fewer statoliths. These results indicate that ephyrae which develop in the microgravity environment of outer space during a flight period of 5-6 days will probably be similarly affected. That is, their graviceptor structures may not be able to synthesize as many statoliths as controls or, indeed, other structures in the graviceptor structure such as hair cells or nerve cells which may not develop normally. These studies using the jellyfish to detect microgravity effects on graviceptor development could lead to an improved understanding of the role(s) of gravity in the differentiation of graviceptor structures on Earth and may ultimately be used to determine if certain levels of g are required to prevent loss of normal development (and function) of graviceptor structures in a microgravity environment. (This kind of information is important for the long-term occupancy of humans and other organisms in space.)

The parabolic flight experiments demonstrated that the brief exposure of ephyrae to different levels of g (0-2) caused behavioral responses. This finding that the ephyrae are very gravisensitive is important because it means that these very simple animals can be extremely useful models to determine how graviceptor structures function to elicit a behavioral response. By comparing results obtained from the jellyfish research with related research in other organisms of different levels of complexity, an understanding of the role of gravity in the evolution of graviceptor systems in animals on Earth may also ultimately be achieved.

EFFECTS OF MUSCLE ATROPHY ON MOTOR CONTROL

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Description of Research

This project concerns the basic mechanisms underlying muscle atrophy. Specifically, the project addresses the issue of the appropriateness of rats raised in conventional-sized cages as experimental models to examine this phenomenon. The project hypothesis is that rats raised in small cages are inappropriate models for the study of muscle atrophy. The experimental protocol involves: (a) raising two populations of rats, one group in conventional (small)-sized cages and the other group in a much larger cage, from weanling age (21 days) through to young adulthood (125 days); (b) comparison of size- and force-related characteristics of selected test muscles in an acute terminal paradigm.

Our results during the previous 2 years dictated our course of action in 1984. In 1982, we raised two populations of male rats and found some significant size- and force-related differences between the two groups. To increase our database, we raised another two populations in 1983, but this time we used female rats. In contrast, the differences we observed initially were absent in the second batch of animals. Consequently, in 1984 we raised four groups of animals with variable combinations of gender and cage size; that is, large cage female, small cage female, large cage male, and small cage male.

Accomplishments

The major findings from our third batch (four-group combination) of animals are:

(1) Gender does not affect the functional response of rats to cage size during rearing.

(2) The morphological and functional consequences of raising rats in a large cage is extremely variable. In some large-cage animals the measurements were substantially different from their small-cage counterparts, while for other large-cage animals there was essentially no difference between the two groups.

Significance of the Accomplishments

Cage size per se during rearing does not appear to determine the morphological and physiological status of rat muscle. It seems, therefore, that we can reject the project hypothesis and thus that small cage-raised rats are reasonable models for the study

of muscle atrophy.

PUBLICATIONS

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Hasan, Z., Enoka, R.M., and Stuart, D.G. The Interface Between Biomechanics and Neurophysiology in the Study of Movement: Some Recent Approaches. In: Exercise and Sport Sciences Reviews, Vol. 13 (ed. by R.L. Terjung). New York: Macmillan Publishing Company, p. 169-234, 1985.

INTRACELLULAR CONTROL OF MUSCULAR ATROPHY AND HYPERTROPHY

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Description of Research

The major physiological function of postural muscles is to develop contractile forces which are directly related to the opposing force of gravity. Microgravity, by reducing the force opposing the forces developed by contracting postural muscles, reduces function, contractile force, and mass of postural muscles. Animal models have been developed to reduce muscular function by reducing muscular activity and can be used to simulate the influence of microgravity on postural muscles. These models include application of casts to limbs, joint immobilization, and hindlimb suspension. While the other models limit muscular function to isometric contractions which permit only force development and not muscular shortening, hindlimb suspension better simulates the conditions of microgravity by permitting passive limb movements and muscular shortening during contraction. Hindlimb suspension is imposed chronically on rats and suspends their rear limbs such that the muscle in the rear limbs are non-load-bearing and have reduced muscular function and activity.

Prior to 1984, research indicated that hindlimb suspension evoked greater atrophy in slow-twitch fibers as compared with fast-twitch fibers. This finding supported the concept that slow-twitch fibers function to maintain posture while fast-twitch fibers function to provide locomotion. During 1984, atrophy of slow-twitch fibers was further investigated to test whether the larger, but atrophying, slow-twitch fibers were transformed into smaller fast-twitch fibers during hindlimb suspension. A second hypothesis which was tested during 1984-1985 was whether prostaglandins were involved with soleus atrophy and hypertrophy. Previous investigations had found that skeletal muscles biosynthesized prostaglandins E_2 and $F_{2\alpha}$, which evoked protein degradation in rat and protein synthesis in rat and rabbit, respectively.

Accomplishments

(1) Hindlimb suspension of rats was found to elicit in the soleus muscle a decrease in the number of slow-twitch fibers, an increase in the number of fast-twitch fibers, but no change in total number of fibers. Further, no evidence was found for a simultaneous complete degeneration of slow-twitch fibers and generation of fast-twitch fibers.

(2) Indomethacin blocks biosynthesis of prostaglandins in muscle. Treatment of rats with indomethacin during recovery from hindlimb suspension inhibited the recovery in weight gain, and hypertrophy of slow- and fast-twitch fibers in soleus muscles.

(3) Release of arachidonic acid from phosopholipids within membranes in muscle controls biosynthesis of prostaglandins in muscle. Content of arachidonic acid within phospholipids of soleus muscles was measured and was found to be changed during soleus atrophy and hypertrophy, which were elicited by hindlimb suspension and recovery from suspension, respectively.

Significance of the Accomplishments

Finding #1 raises the possibility that hindlimb suspension evokes a transformation of individual slow-twitch fibers of the soleus muscle into small fast-twitch fibers. This possibility of transformation of fiber types contradicts a widely held concept that a selective decline in the number of slow-twitch fibers is evoked by muscular inactivity, but is consistent with evidence for transformation of fiber types by changes in the pattern of motor neuronal activity. Consequently, this possibility that muscular inactivity elicits fiber-type transformation may help to unify the mechanisms responsible for muscular changes stimulated by both muscular inactivity and neural activity.

Findings #2 and #3 raise the possibility that muscular atrophy and hypertrophy may be controlled at the cellular level by controlling prostaglandin biosynthesis. Prostaglandins are paracrine agents which evoke their major influences in the cells where they are biosynthesized. Whereas steroids are currently used by athletes to augment muscular hypertrophy and other hormones, such as insulin, are currently being investigated to control muscular size, recent evidence indicates that prostaglandins mediate the influence of steroids and insulin on muscular size. Controlling muscular atrophy and hypertrophy at the cellular level with prostaglandins may be advantageous as compared with the use of hormones which have broader systemic influences and may have undesirable side effects.

PUBLICATIONS

Templeton, G.H., Padalino, M., Manton, J., Glasberg, M., Silver, C.J., Silver, P., DeMartino, G., LeConey, T., Klug, G., Hagler, H., and Sutko, J.L. Influence of Suspension Hypokinesia on Rat Soleus Muscle. Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology 56: 278-286, 1984.

Templeton, G.H., Padalino, M., Manton, J., LeConey, T., Hagler, H., and Glasberg, M. The Influence of Rat Suspension-hypokinesia on the Gastrocnemius Muscle. Aviation, Space, and Environmental Medicine 55: 381-386, 1984.

SKELETAL MUSCLE METABOLISM IN HYPOKINETIC RATS

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Description of Research

This work focuses on understanding the influence of lack of weight-bearing (unloading) on leg muscles as occurs under weightless conditions during spaceflight or as a result of extended bed rest following injury or illness. The most noticeable response is the loss of mass by certain muscles followed by loss of muscle function when this muscle-wasting becomes excessive. To study the biochemical changes associated with this muscle-wasting, we use rats which are allowed to walk on their forelimbs but not their hindlimbs. The hindlimbs remain free-moving but do not bear any of the animal's body weight. In previous years, the work had shown significant changes in the breakdown or production of certain biochemical substances essential to the normal functioning of muscle.

When animals are subjected to hindlimb unloading, the blood levels of certain hormones (e.g., glucocorticoids) rise during adaptation to walking only on their forelimbs. Hence, during 1984, the research focused on the question of to what extent do elevated glucocorticoid hormones account for the changes in use or production of protein and amino acids in unloaded muscles? Other questions addressed during the year included: (a) What changes in use of glucose are associated with muscle unloading? (b) What role might insulin play in the known accumulation of glycogen (storage form of glucose in muscle) in soleus leg muscle of animals subjected to weightlessness or unloading? (c) What is the effect of unloading on the ability of muscle to take amino acids into the tissue? These studies were conducted in unloaded soleus and extensor digitorum longus muscles. These muscles were chosen because the former but not the latter leg muscle is sensitive to the reduced activity in unloading. Hence the extensor digitorum longus muscle allows one to determine whether any given effect may be due to unloading per se or to factors (e.g., hormones) in the blood.

Accomplishments

(1) Biochemical changes of muscle (i.e., extensor digitorum longus), which grow normally in unloaded hindlimbs, can be prevented by removing the animal's adrenal glands (source of glucocorticoid hormones).

(2) Certain biochemical changes of muscle which loses size with unloading (i.e., soleus) are due to hormone secretions of the adrenal gland. These changes included: slower production of muscle protein and greater capacity to produce glutamine, an

amino acid.

(3) In the atrophied (wasted) soleus muscle, faster breakdown of protein and slower production of glutamine are due solely to the effects of lack of weight-bearing on the muscle.

(4) Unloaded soleus muscle uses more glucose because insulin is better able to facilitate the entry of glucose into this muscle than into the normal muscle.

(5) The conversion of glucose to glycogen (storage form) is much more rapid in the unloaded soleus muscle than the normal muscle when insulin is present. No such difference is seen for conversion of another substance (pyruvate) to glycogen.

(6) Insulin has a decreased ability to facilitate the entry of amino acid into unloaded soleus muscle.

Significance of the Accomplishments

Finding #1 shows that even minor stress must be considered in interpreting any biochemical changes that occur with physiological adaptation. As noted above, analysis of the extensor digitorum longus muscle provided us with an ideal control for testing for effects other than those due to unloading. Based on these observations, it was then possible to ascertain which changes in the soleus muscle were not due to unloading per se (Finding #2) and which were a direct consequence of unloading and the resulting muscle atrophy (Finding #3). It is these latter biochemical changes which we plan to examine in muscles to be obtained from rats flying on the Spacelab (SL)-3 mission.

It is surprising that glucose use is elevated in the soleus muscle with unloading, due to increased stimulation by insulin (Finding #4), since a muscle being used less should require less glucose. This latter consideration may show why Finding #5 was obtained. Since the muscle is not using the excess glucose, this glucose is probably stored, instead, as glycogen. Such findings will be important in understanding better how the use and storage of glucose is controlled in muscle.

Finally, Finding #6 shows the opposite effect of insulin as that seen in Finding #4, that is, amino acid entry into the unloaded muscle is less responsive to insulin than is the entry of glucose. This finding indicates the mechanism for the effect of insulin is different in each case. Since little is known about the mechanism of insulin action, these data could be useful for examining this problem.

PUBLICATIONS

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EFFECTS OF SIMULATED WEIGHTLESSNESS ON MEIOSIS, FERTILIZATION, AND EARLY EMBRYONIC DEVELOPMENT OF MICE

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Description of Research

This research is focused on understanding the role of gravity in mammalian development and reproduction. Our emphasis is twofold--first, to understand how basic processes of mammalian development and differentiation have been affected by the 1 g environment of Earth; and second, to examine the effects of an altered gravitational environment on these processes. One aspect of spaceflight, namely, a microgravity environment, is the parameter being studied. The specific aspects of reproduction under investigation, in an in vitro culture system, are meiosis, fertilization, and early embryogenesis, using mouse as a model system. To simulate a microgravity environment, cells in the developmental stage of interest are rotated on an axis perpendicular to the gravity vector, using a clinostat. Our previous work involved adapting a clinostat for use with mammalian tissue culture cells. In the last year, research was directed to examining the effects of clinostat rotation on the meiotic maturation of the mouse oocyte (which, when mature, is termed an ovum or egg). This maturation process involves the breakdown of the cell's nucleus and separation of the chromosomes in preparation for fertilization by the sperm.

The question we wished to address was to what extent would reorientation of cells, relative to the gravity vector during this maturation process, affect (a) the morphology of cells, (b) the ability and efficiency of the nuclear breakdown, and (c) the movement and final orientation of the chromosomes. In addition, studies were begun examining the process of fertilization of eggs by sperm, again under the clinostat rotation system.

Accomplishments

The major findings from this research to date are:

(1) Oocytes are capable of entering into the process of meiotic maturation while cultured in the clinostat rotation system. This conclusion is based on the observation that the frequency of breakdown of the germinal vesicle (nucleus) was similar between experimental and control cultures at rotation rates ranging from 1/4 to 100 rpm.

(2) Reorientation of the cells during maturation can result in effects on the normal progression of the separation of the chromosomes. After 14-16 hr in culture, mouse oocytes should be in the Metaphase II stage of meiosis, that is, with the first

stage of the chromosome separation (or reduction division) complete. This normal progression was observed in experimental and control rotations at 1/4 to 30 rpm. In contrast, at 100 rpm, the rotated oocytes revealed a significant decrease in the number of oocytes that achieved the Metaphase II stage.

(3) Preliminary studies are underway examining the next step in the developmental process--fertilization. To date, it is clear that fertilization of rotated ova can be accomplished and the progression of fertilization is indistinguishable between the experimental and control cultures at the rotation speeds examined thus far, 1/4 and 100 rpm. Further studies will be necessary to establish if the efficiency of sperm binding and other very early events in the process of fertilization are normal.

Significance of the Accomplishments

Finding #1: The observation that oocytes rotated on the clinostat could efficiently resume meiosis was of interest on several levels. First, it was consistent with other observations that this cellular event, that is, the breakdown of the cell's nucleus, occurs very rapidly after release from the ovary. Therefore, we predicted that this event indeed not be expected to respond to altered gravity orientation. Second, it suggested that this cellular event is not a particularly gravity-sensitive event. This, of course, should be confirmed in in vivo and in the true microgravity of space.

Finding #2: In contrast to the absence of effects on breakdown of the nucleus, the observations suggest that later events of this cellular process, namely, chromosome separation (or disjunction), were indeed affected by reorientation to the gravity vector on a clinostat. Such an inhibition would affect the capability of the egg to then be fertilized and would therefore be predicted to affect the reproductive potential of the cells. The effects of chromosomes in the meiotic cell cycle are of particular interest in light of the results of several flight experiments in which effects have been observed in the process of cell division.

Finding #3: Finally, our experiments on fertilization under clinostat rotation suggest that once fertilization has occurred, the male and female pronuclei form normally. However, additional experiments are needed to ascertain if the overall fertilization efficiency and timing of fertilization events are comparable in the experimental and control conditions. These aspects are critical to ensuing development and successful reproductive outcome.

PUBLICATIONS

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Wolgemuth, D.J. and Grills, G.S. Effects of Clinostat Rotation on Mouse Meiotic Maturation *in vitro*. *Physiologist* 27(6, Suppl.): S99-S100, 1984.

SPECIAL ACTIVITIES

RESEARCH ASSOCIATE PROGRAM IN SPACE BIOLOGY

The NASA Shuttle Program currently provides a unique opportunity to conduct biological research in outer space and to continue relevant ground-based Space/Gravitational Biology research. To maximize the potential for Space/Gravitational Biology as an emerging discipline, there is a need to develop a cadre of scientists who are interested in working in this field. The Research Associate Program in Space Biology utilizes a competitive grant process and awards are made to the more promising applicants. It is anticipated that these scientists will develop their research careers in the newly evolving discipline of Space/Gravitational Biology.

The grant is designed to make awards on January 1 and July 1 of each year, following semiannual announcements. An Award Committee reviews the proposals and recommends appointments. The awards are for a 1-year period, with the possibility of renewal for a second year. The program began on June 1, 1980, and since then 44 awards have been made. The recipients have been 27 scientists, including zoologists, developmental biologists, botanists, and physiologists (animal and plant). Seventeen of these individuals have received a second year of funding. These scientists work in NASA-funded laboratories or laboratories that can provide the necessary facilities and environment for specialized Space/Gravitational Biology projects. Originally (June 1980) there were 19 laboratories participating. Presently (August 1985), there are 46 laboratories participating.

In addition to the salary stipend, the awardees are encouraged to attend and present papers at two national meetings: 1) the annual AIBS/NASA meeting, and 2) a national society meeting of their choice.

The success of the program is readily measured in terms of the quality and quantity of publications as well as the job opportunities and placements of the Research Associates. Individuals have obtained positions in college or university settings and in research laboratories. A large number of publications have resulted from this program.

The grant is administered through the University of Louisville, Louisville, Kentucky. Dr. X.J. Musacchia, Dean of the Graduate School, is the Project Director and Science Advisor.

MEETINGS

Organized or Participation by the Space/Gravitational Biology Program:

- "Plant Gravitational and Space Research Workshop," Rosslyn, Virginia, USA, April 30-May 2, 1984.
- "Animal Development Workshop," Rosslyn, Virginia, USA, May 2-4, 1984.
- "55th Annual Meeting of the Aerospace Medical Association," San Diego, California, USA, May 7-10, 1984.
- "25th Plenary Meeting of the Committee on Space Research (COSPAR)," Graz, Austria, June 23-July 7, 1984.
- "14th Annual Intersociety Conference on Environmental Systems," San Diego, California, USA, July 16-18, 1984.
- "Annual Meeting of the American Society of Plant Physiologists," University of California at Davis, Davis, California, USA, August 12-17, 1984.
- "35th Annual Meeting of the American Physiological Society," Lexington, Kentucky, USA, August 26-31, 1984.
- "6th Annual Meeting of the IUPS Commission on Gravitational Physiology," University of Lausanne, Lausanne, Switzerland, September 18-21, 1984.
- "9th Annual Symposium of the NASA Gravitational/Space Biology Program," Harpers Ferry, West Virginia, USA, November 6-9, 1984.
- "Animal Gravity-sensing Systems Workshop," Pacific Grove, California, USA, February 24-26, 1985.
- American Society for Gravitational and Space Biology founded November 1984; first meeting scheduled for October 1985.

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
NASA TM-88379		

4. Title and Subtitle	5. Report Date
1984-85 NASA Space/Gravitational Biology Accomplishments	December 1985
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
Thora W. Halstead, F. Ronald Dutcher, and Linda G. Pleasant, Compilers	
	10. Work Unit No.
9. Performing Organization Name and Address	
NASA Office of Space Science and Applications Washington, D.C. 20546 and The George Washington University, Washington, D.C. 20037	11. Contract or Grant No. NASW-3165
	13. Type of Report and Period Covered
12. Sponsoring Agency Name and Address	Technical Memorandum
National Aeronautics and Space Administration Washington, D.C. 20546	14. Sponsoring Agency Code EBR

15. Supplementary Notes

Thora W. Halstead: NASA Office of Space Science and Applications, Washington, D.C.
F. Ronald Dutcher and Linda G. Pleasant: The George Washington University, Wash, D.C.
For previous edition, see NASA TM-86654

16. Abstract

The report consists of individual technical summaries of research projects of NASA's Space/Gravitational Biology Program. The summaries for each project include a description of the research, a listing of the accomplishments, and an explanation of the significance of the accomplishments. Bibliographies for each project are also included.

17. Key Words (Suggested by Author(s))		18. Distribution Statement
Gravity	Animal	Unclassified - Unlimited
Space	Plant	
Weightlessness	Hypogravity	
Simulated weightlessness	Hypergravity	
	Life sciences	Subject Category 51

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	144	A07

For sale by the National Technical Information Service, Springfield, Virginia 22161

NASA-Langley, 1985

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